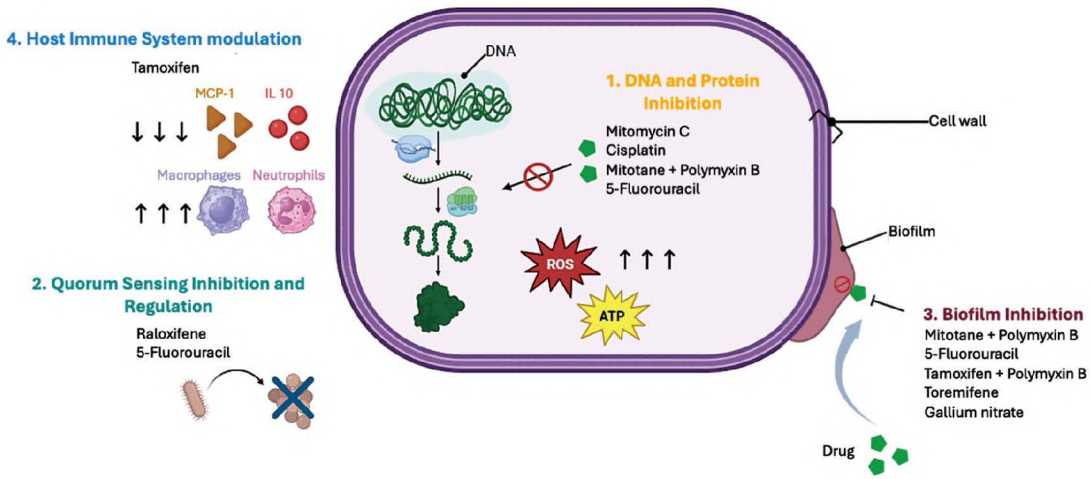
Drug Repurposing

Chemistry, Biochemistry and Red Biotechnology



Editor
Munishwar Nath Gupta

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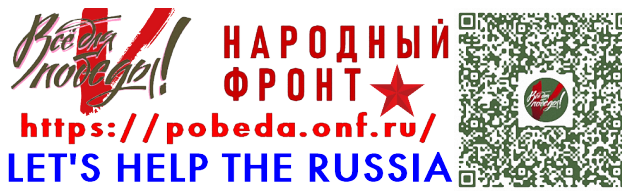

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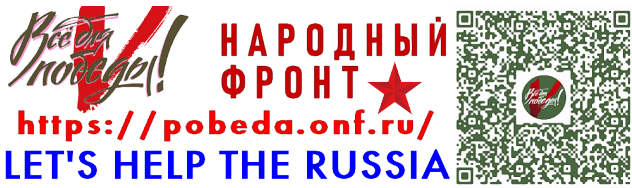

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Preface

Among various branches of biotechnology, red biotechnology deals with healthcare and pharmaceuticals. Hence, it encompasses drug discovery and drug repurposing. Drug repurposing is also called drug repositioning, drug reprofiling, drug switching, drug indication expansion, drug indication shift, and drug redirecting. As most of these terms imply, it is essentially about finding new therapeutic uses for an existing drug. Selective optimization of side activities (SOSA) or structural modification of an existing drug to improve it for an approved purpose is generally not considered drug repurposing.

As discussed in this book, the primary USP of drug repurposing is that it can short-circuit the lengthy and costly process of drug discovery and drug approval by the regulatory agencies. That also becomes critical when we suddenly have to face an endemic or a pandemic like COVID-19.

My interest in drug repurposing arose from my interest in the biological specificity (or lack of it!) of proteins. Drug repurposing, essentially an outcome of non-specificity of drug-target interactions, looked like a case of promiscuity observed in the case of affinity pairs, which I had been exploiting for protein bioseparation. We had observed accidentally that many naturally occurring or synthetic polymers bind to proteins exhibiting pseudo-affinity. Almost all drug repurposing discoveries have been serendipitous and unpredictable. The next step in such cases is to decode why and how such interactions occur. That is where we are at present in the area of drug repurposing. Such efforts include improving predictability which also necessarily involves understanding the chemistry and biochemistry of the phenomena. That, as the title indicates, is the focus of the present book.

The first chapter on polypharmacology discusses how drug repurposing rests upon the non-specific nature of drug-target interactions. So, drug promiscuity and protein promiscuity are the reverse sides of the same coin. The drug repurposing in the cases of neurodegenerative diseases and psychiatric disorders is discussed in chapters 2 and 3, respectively. Chapter 4 discusses the biochemical aspects of cancer and efforts to exploit drug repurposing in a rational fashion for treating cancer. Multi-drug resistance is a serious problem when using antibiotics to treat infectious diseases. TB is an important example of that. So, chapters 5th and 6th should be read in that order. Chapter 6 considers drug repurposing for helminthic infections, which are also not easy to treat. As the perusal of these chapters will reveal, drug repurposing has not always been hugely successful. It is our failures rather than successes that teach

us more. Hence, the book intentionally focuses on challenges in drug repurposing rather than highlighting success stories of drug repurposing, which have often been emphasized when discussing this approach.

The last chapter discusses "vaccine repurposing." Lest we overlook, the use of anti-serum against cowpox as a vaccine for smallpox by Jenner was a case of vaccine repurposing. This chapter is an outlier as vaccines are not discussed in the context of drug repurposing, although vaccines meet the statutory definition of a drug.

One notable omission in this book is the discussion on repurposing drugs for rare diseases. Given their small market sizes, orphan drugs as such are not attractive products for pharma industries.

Numerous databases useful for drug repurposing are available and are listed by Roy and Gupta (Biol. Rev. 96: 205-222, 2021). There is also a freely available Drug Repositioning Platform (http://www.way2drug.com/dr).

The chapters have a bit of repetition of basic information about drug repurposing, but it is useful to view that in the context of different diseases. This will also allow a reader to not necessarily read the chapters chronologically.

I thank all the authors for agreeing to contribute to the individual chapters. I thank Mr. Raju Primlani of Science Publishers (an imprint of CRC) for his patience, support, and advice on many issues. I also thank other CRC team members who worked behind the scenes to produce this book.

Drug repurposing will be a much more powerful toolbox when it no longer depends on serendipity. Hence, looking at this approach through the twin windows of chemistry and biochemistry is critical. Technology is a real-life application of science. So, this sector of red biotechnology will profit from gaining insights from chemical and biochemical sciences.

I hope the book will be useful to both active research workers engaged in drug discovery programs and students of biological and medical sciences.

November 7, 2024 **Munishwar Nath Gupta**

Delhi, India

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CHAPTER

1

Polypharmacology: Drug Promiscuity Enabled by Protein Promiscuity

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Introduction

The rational drug discovery strategies were initially focused on identifying molecules capable of specific binding to an intended target. These targets were either enzymes (inhibited by the screened-in molecules) or extracellular/intracellular protein receptors. This approach centers on the biological specificity of proteins, which is the cornerstone of biological sciences. The concept of the biological specificity of proteins includes recognition of the existence of "affinity pairs" (Mattiasson 1988; Roy and Gupta 2000), which are exemplified by the coenzyme NAD^+-dehydrogenases, lectin-sugars, and biotin-avidin pairs. So, there is a reciprocity of specificity in affinity interactions among the pairs. In the context of drugs, we can include drug-receptor (or target as a more general term) as the affinity pair.

The notion of drug specificity was enshrined as the magic bullet ("Zauberkugeln," which is a drug that targets a specific disease-causing agent without harming healthy tissues) concept proposed in 1907 by Paul Ehrlich (1854–1915) (Ehrlich 1908; Witkop 1999). The drug would bind to a specific target because the target receptor or enzyme is specific to that drug molecule. The magic bullet concept underlined the specificity inherent in this affinity pair and implied the particular action of the drug on the target.

Over the last few decades, the narrative of biological specificity has undergone drastic changes. Not only proteins have turned out to often lack biological specificity,

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but this lack of specificity, as illustrated by their promiscuity and moonlighting behavior itself, seems to be critical for protein evolution and their function in numerous biological processes (Gupta and Mukherjee 2013; Gupta et al. 2020; Gupta and Roy 2021).

The magic bullet concept is, in reality, a lot less magical. Polypharmacology refers to a drug molecule binding to more than one kind of receptor and such interactions prove therapeutically useful (Jalencas and Mestres 2013; Kabir and Muth 2022). This lack of specificity, called drug promiscuity, again originates in the promiscuous nature of drug interaction with the corresponding receptors/enzymes. It is like a free/open marriage, where both partners are promiscuous.

Therefore, for a clear understanding of polypharmacology at the molecular level, it is desirable to understand how biological specificity has evolved first. Hence, this chapter on polypharmacology next delves into the shifts in the classical paradigm of structure and function relationships in proteins.

The Nature of Biological Specificity (or Lack of It)

Quite early, it was realized that in many cases, biological specificity is not absolute because there is no one-to-one relationship between a ligand and the protein to which it binds (Gupta 2002; Gupta and Roy 2018). Thus, classical enzymology talked of group-specific ligands. Coenzyme NAD^+ binds to several dehydrogenases, mannose will bind to several lectins, and an antigen can bind to several (polyclonal) antibodies. The cross-reactivity of antibodies was an exciting observation of those early times. Yet, these facts did not override the notion that there is a binding site (called active site in the case of enzymes) that has a unique specificity.

Biological specificity distinguishes biology from chemistry. It was understood that these high-affinity interactions are biologically relevant, even if not completely understood in some cases. The twin blow to this notion came from the reports that metal ions (particularly those of Cu, Ni, Zn, and Fe) along with textile dyes (notably cibacron blue) can serve as excellent affinity ligands in affinity-based bioseparation of proteins (Sulkowski 1985; Roy and Gupta 2000; Labrou 2003). While with dyes, there were some attempts to show that their similarity in structures with biologically relevant ligands (substrates, inhibitors, etc.) may be responsible, metal ions binding to proteins dealt a deathblow to the assumption that all affinity interactions are biologically relevant or involve a specific site on the proteins. Later, a discovery that peptides identified in peptide libraries are capable of reasonable selectivity in binding toward all sorts of proteins clearly established that the biological specificity of proteins was a lot more complex and was yet to be completely understood.

The next strand of this story is the discovery of protein promiscuity and moonlighting (Gupta and Uversky 2023a; Gupta and Uversky 2023c; Gupta and Uversky 2023e). The latter, in fact, erased the distinction between enzymes and other non-catalytic proteins. As a classic example, an eye protein crystallin was shown to have some well-known activities in other tissues/organs. For protein promiscuity, a well-known example is that of lipase. The enzyme, which digests fats/oils, can also show esterase, esterification, and transesterification activities. Even more strikingly,

it can catalyze C-C bond formation like aldolases. That raised the question about the well-known EC classification of enzymes (Kapoor and Gupta 2012).

A remarkable finding, which is curiously seldom mentioned in the context of the biological specificity of proteins, is the technique of bioimprinting. It has been shown that if a protein in the presence of a large concentration of a ligand, which can form enough weak bonds with the protein, is rigidified (by dehydration) either by lyophilization or precipitation with an organic solvent, the protein retains the imprint (acquires and keep the binding site for the ligand) even when the ligand is removed. In some cases, imprinting with a co-substrate improved the catalytic power of the enzyme (Majumder and Gupta 2011; Mukherjee and Gupta 2015a; Mukherjee and Gupta 2015b; Mukherjee and Gupta 2016).

Yet another blow to the classical notion of biological specificity was the discovery that proteins need not possess a pre-formed binding site (Uversky and Dunker 2013). The discovery of intrinsic disorder, which turned out to be as important as "structure," has been one of the most defining moments in the history of protein sciences (Uversky 2013; Gupta and Uversky 2023d; Gupta and Uversky 2024). The binding site is formed in intrinsically disordered proteins (IDPs) or intrinsically disordered regions (IDRs) of proteins upon interaction with a partnering ligand. It is also remarkable that molten globules, which are significantly more disordered than the native states of many proteins, still retain a reasonable level of biological activity in quite a few cases (Gupta and Uversky 2023b). In view of the growing footprint of intrinsic disorder in drug design and, in particular, drug promiscuity, and given that it is still not covered in most standard textbooks on enzymology, let us discuss this concept in some detail.

Intrinsic Disorder and Absence of Pre-formed Binding Sites

The protein structure-function paradigm has been the basis of our views on what all proteins do in biological systems and what applied enzymologists/biotechnologists try to make them do in the laboratory/industry. Biochemists inherited this belief from organic chemists. As we know, one of the famous organic chemists of his era, Hermann Emil Fischer (1852–1919), gave us the "lock and key" hypothesis, which firmed up the notion of steric fit between two structures – enzyme and the substrate. It has served biologists well. The success of X-ray crystallography in determining structures of so many proteins and their complexes with canonical ligands (specific keys) created a strong and credible narrative on "pre-formed binding sites" in native structures being the corresponding keyholes. Denature the protein, disturb the structure, and the function is diminished/abolished.

Sure, somewhere down the line, the "hands-and-glove" model was considered a more realistic one than "lock and key" (Koshland 1958). This induced fit hypothesis by Daniel E. Koshland Jr. (1920–2007) had a rough time and was even ridiculed for quite a few years (Koshland 1993). Ultimately, protein flexibility/dynamics became accepted, and protein underwent a transition from a rigid lock to a (somehow) flexible glove, which is able to adjust to the shape of the hand (ligand). Structure and

function were still the guiding philosophy, except the structure was flexible. Still, the necessity of a unique native structure was not questioned.

The aforementioned magic bullet ("Zauberkugel"), the concept of Paul Eherlich (Huber and Bennett 1983; Witkop 1999; Heynick 2009), which has been the cornerstone of drug discovery and design for decades, was a part of the same narrative. The magic bullet concept implies that the drug is specific to the receptor to which it binds. Such absolute specificities were visualized in terms of a rigid 3-D structure of a keyhole fitting in with the rigid key.

Slowly but surely, cracks started appearing in this beautiful edifice centered on the idea of structure being essential for function. As early as the 1980s, it was realized that the protein regions where X-ray diffraction does not detect structure may have functional significance (Huber and Bennett 1983). Transcription factors were found to rely upon "acid blobs or negative noodles," a metaphor used to describe ill-defined structures, for their biological activity (Sigler 1988). Brian W. Pontius pointed out that unstructured domains may have an advantage in increasing the rates of specific macromolecular association (Pontius 1993). By the end of the last century, it became clear that not all biologically functional proteins have rigid structures, and the related recognition of the importance of the biological function of intrinsic disorder created a new paradigm of structure-independent functions. It helped that the use of new tools to look at proteins started slowly complementing information from X-ray diffraction (Uversky and Dunker 2010; Uversky and Longhi 2011; Uversky and Dunker 2012a; Uversky and Dunker 2012b; Uversky 2015).

The irony of X-ray diffraction as a tool in studying proteins was that if it did not detect structure, it could not assess the biological significance of that protein region. NMR turned out to be a powerful tool for studying intrinsic disorders (Jensen et al. 2009; Felli and Pierattelli 2012; Kosol et al. 2013; Brutscher et al. 2015; Kurzbach et al. 2015; Salvi, Abyzov and Blackledge 2017; Adamski et al. 2019; Hartlmüller et al. 2019; Mateos et al. 2019; Leeb and Danielsson 2020; Nielsen and Mulder 2020). The paramagnetic relaxation enhancement approach enables measurements of long-range interactions. This is valuable, as IDPs have transient long-range contacts (Hartlmüller et al. 2019; Mateos et al. 2019). NMR enabled the observation of the dynamics of disordered protein segments on a time scale ranging from ps to ns (Salvi et al. 2017). So, one was no longer confined to assessing protein flexibility from the B-factors of structures derived from X-ray diffraction. Heteronuclear Overhauser effects, in combination with the NMR relaxation technique, were able to characterize peptide backbone dynamics of IDPs (Gondelaud et al. 2023).

Another experimental technique that has proved valuable in determining the compactness of a protein is synchrotron-based small-angle X-ray scattering (SAXS). The theoretical framework to distinguish ordered structure and unfolded/disordered based on the data obtained by SAXS exists (Uversky and Dunker 2010; Uversky and Longhi 2011; Uversky and Dunker 2012a; Uversky and Dunker 2012b; Uversky 2015; Gondelaud et al. 2023).

It was also recognized that IDPs/IDRs differ from the random coil polypeptides, as disordered regions show preferences for well-defined structures described well by the phrase "dynamic secondary structure content" (Chemes et al. 2012). These dynamic secondary structures can be stabilized (at least to some degree) in the

presence of specific ligands. This enables using circular dichroism (CD) spectroscopy to study the conformations and conformational transitions of IDPs. Far-UV CD was useful in this and for estimating the residual secondary structure content of IDPs (Gondelaud et al. 2023).

Results reported in the literature show that the secondary structure of IDPs in their bound complexes with partnering ligands is transiently formed. Iesmantavicius et al. (2014) studied the interaction of the intrinsically disordered domain of the activator for thyroid hormone and retinoid receptor (ACTR), which is known to fold upon binding to the nuclear coactivator binding domain (NCBD) of cyclic AMP response element binding (CREB) protein. They used NMR and fluorescence-based stopped-flow kinetics analysis to look at the effect of mutations affecting the secondary structure content of ACTR on the binding kinetics (Iesmantavicius et al. 2014). Their results indicated that the secondary structure content of helix 1 affected the binding kinetics. These observations supported the idea that the pre-formed secondary structure has a clear role in facilitating the interaction of IDP/IDRs in their interaction with other proteins.

Intrinsic Disorder, Aggregation, and Conformational Diseases

One area that accelerated the research on intrinsic disorder is the discovery that several diseases are caused by protein misfolding and resulting aggregation (Dobson 2003; Barral et al. 2004; Dobson 2006; Chiti and Dobson 2006; Luheshi et al. 2008; Vendruscolo et al. 2011; Hipp et al. 2014; Hartl 2017; Chiti and Dobson 2017; Dobson 2017). Uversky et al. (2008) wrote one of the early comprehensive reviews on how IDPs/IDRs are involved in numerous diseases, which arise because a protein changes its functional conformation to a misfolded one (Uversky et al. 2008). Misfolding can lead to fibril formation, loss of biological function, and assuming a toxic function. "Misfolding and misfunction can originate from point mutation(s) or result from an exposure to internal or external toxins, impaired posttranslational modifications (PTMs) (e.g., phosphorylation, advanced glycation, deamidation, and racemization), an increased probability of degradation, impaired trafficking, lost binding partners, or oxidative damage. All these factors can act independently or in complex associations with one another" (Uversky et al. 2008). Many of these protein-misfolding diseases are caused by the formation of amyloids. These amyloid fibrils are stable aggregates that get deposited in the organs and tissues. They are characterized by the "core cross β-sheet structure." It is interesting that native structures or disordered regions can all end up as amyloids. We know now that the earlier notion of only certain proteins being able to form amyloids was incorrect and that almost any protein can form amyloids, albeit under conditions that may differ or lack physiologically relevance. As a characteristic of many protein aggregations, amyloid formation is mediated by the unfolding of the polypeptide (for structured proteins) or stabilization of an unfolded structure (in the case of IDPs) (Uversky and Fink 2004). The initially formed soluble aggregates, in some cases, turn into insoluble amyloid structures. The process does not occur in isolation and depends upon several metabolic changes and/or disturbances in the signal transduction and other regulatory processes of the cell.

Uversky et al. (2008) have discussed some interesting examples of IDPs to illuminate why IDPs are predominantly associated with diseases (Uversky et al. 2008).

An increase in the α-fetoprotein (AFP) content in adult sera is a marker of several pathologies, including cancer (Abelev et al. 1963; Abelev 1971; Abelev 1989; Abelev and Eraiser 1999; Gillespie and Uversky 2000; Terentiev and Moldogazieva 2013; Wu et al. 2022). AFP and human serum albumin (HSA) are structurally similar (see Figure 1).

HSA binds to more diverse metabolites but with much less binding affinities. Unlike HSA, upon removal of ligands, AFP gets converted into a molten globular

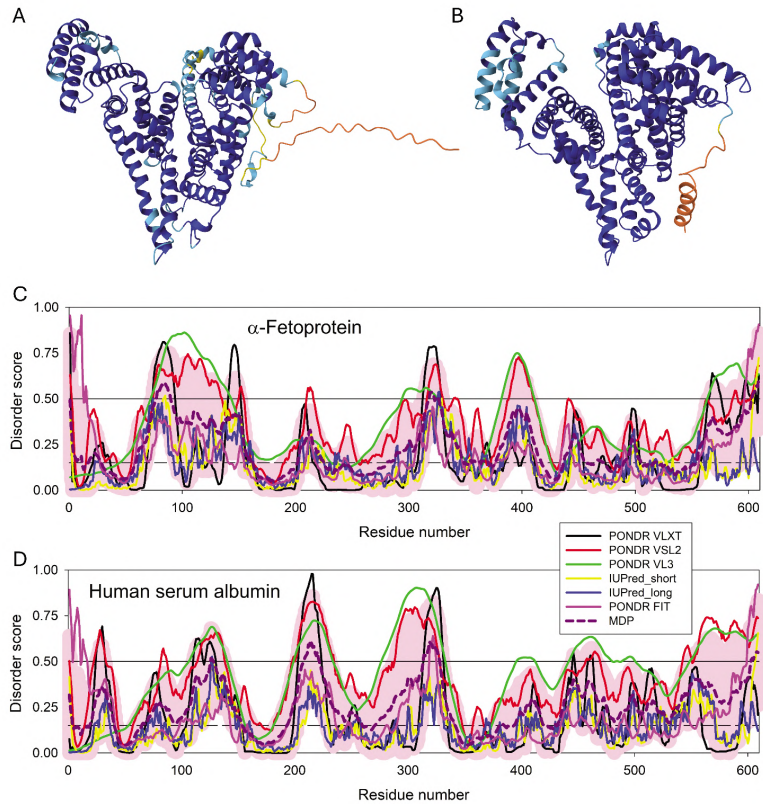

Figure 1. Order and disorder in human AFP (UniProt ID: P02771) and HSA (UniProt ID: P02768). Plots A and B show 3D structures modeled for AFP and HSA by AlphaFold. Structure is colored based on the AlphaFold-generated per-residue confidence score (pLDDT) between 0 and 100, where regions with very high confidence score (pLDDT > 90) are shown by blue color, confidently predicted regions (90 > pLDDT > 70) are shown by cyan color, whereas regions predicted with low (70 > pLDDT > 50) and very low confidence (pLDDT < 50) are shown by yellow and orange color, respectively. Some regions with low pLDDT may be unstructured in isolation. Plots C and D show intrinsic disorder profiles of human AFP and HSA generated by the RIDAO platform (Dayhoff and Uversky 2022). Here, residues with disorder scores above the threshold of 0.5 (this threshold is shown by thin black solid line) are considered disordered, whereas residues with disorder scores between 0.15 (this threshold is shown by thin dashed black line) and 0.5 are considered flexible. ⏎

(disordered) form (Uversky et al. 1997a; Uversky et al. 1997b), which favors its immunosuppressive activity. An interesting observation is that elevated levels of AFP were found to be associated with the presence of acute-phase serum amyloid A, which is known to be a marker of testicular cancer (Yamada et al. 2013). So, while AFP may not form amyloids, it seems to be a part of the network, which includes proteins that do form amyloids. Also, we generally associate disorder-to-order transition with a biologically relevant interaction involving an IDP. In this case, the order-to-disorder transition, which leads to ligand-free AFP becoming a molten globule, has immunosuppressing activity.

The other example is given by p53, which is also called tumor protein 53, cellular tumor antigen 53, or transformation-related protein 53. This protein is involved in repairing damaged DNA and "the integration of cell birth/death signaling pathways with DNA damage checkpoints" (Park et al. 2016). It has two long IDRs. The mutations, which lead to loss of p53 function, are associated with cancers of the colon, lung, esophagus, breast, liver, brain, and hemopoetic tissues. In fact, p53 regulates > 150 genes by interacting with numerous proteins, with a large number of such interactions being dependent on its IDRs (Oldfield et al. 2008; Uversky et al. 2008; Uversky 2016). Also, a large number of posttranslational modifications (PTMs) occur within the p53 IDRs (Uversky 2016). The functional roles of different PTMs outlined in a recent review (Marei et al. 2021) are summarized in Figure 2. The molecular-level understanding of how the mutants of p53 help oncogenesis has been discussed by (Marei et al. 2021).

To conclude, Hazy and Tompa (2009) pointed out that while the IDP binds to their ligands by folding the short disordered sequences or domains, induced folding has some distinct features in these cases. Firstly, the fuzziness/floppiness in the rest of the IDP/IDR can often be retained. Secondly, as their interactions can be multiple simultaneously in a promiscuous fashion, the induced folding is different at different sites depending upon the ligand. The point is that a structured protein cannot achieve this even by leveraging induced folding (Hazy and Tompa 2009).

Chandra et al. (2023) have recently pointed out that, unlike structured globular proteins, the effect of mutations on the biological function of IDPs has been less investigated. They carried out deep mutational scanning of a mutant library of CcdA displayed on yeast surfaces and found that the effect of mutations on the binding (with the partner ligands) is rather localized, presumably as the interacting surface is much larger in the case of IDPs. This enabled them to "use a single rationally chosen ligand concentration" to quantify the relative binding constants of the mutants without resorting to a titration-based approach (Chandra et al. 2023).

"Interestingly, the highly promiscuous drug targets are enriched in disorder when contrasted with the overall set of drug targets and the low promiscuity drug targets (p-value < 0.0001), while their disorder levels are comparable to the possibly druggable proteins. This coincides with the observation that disordered regions are capable of interactions with multiple partners…. Our results suggest that although low disorder amounts are a strong marker for the current drug targets, the set of possibly druggable proteins includes large amounts of disorder. In fact, the disordered proteins may become the key to unlocking a substantial portion of yet to be discovered druggable targets" (Ghadermarzi et al. 2019).

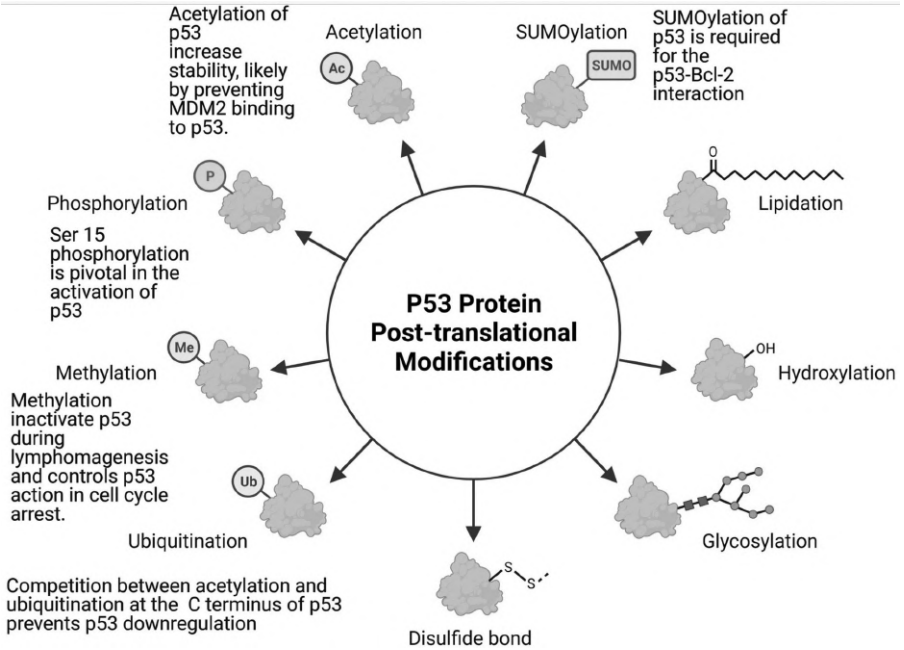

Figure 2. Phosphorylation, acetylation, methylation, glycosylation, ubiquitination, and sumoylation occur in different regions of the p53 protein and play a crucial role in regulating p53 stability and localization in response to genotoxic stress. The p53 ubiquitination mostly occurs at its C terminus, where acetylation also occurs during cell stress, and competing with ubiquitination prevents p53 downregulation, thus enhancing p53 stability. Reproduced from Marei et al. (2021) under the CC 4.0 attribution license (Marei et al. 2021). ⏎

Essentials of Polypharmacology

It is well-established that almost every drug has some side effects. Drug discovery research aims at minimizing such side effects. The various phases of the drug trials are aimed at ensuring that the benefits outweigh the risks. These side effects or their intensity are not the same for all persons. In many cases, it also varies with gender, age, or conditions, such as pregnancy or any co-morbidity. The side effects arise as a drug molecule can bind to proteins from multiple steps in a metabolism or signal cascade or steps of a different metabolic pathway or a signal cascade. Polypharmacology essentially attempts to take advantage of this. It is thus an approach based upon the "single drug for multiple diseases" concept. That is not to say that side effects are suddenly advantageous. However, the existence of side effects leads researchers to identify additional useful targets. This is especially valuable for complex diseases like cancer or neurodegenerative diseases, which involve changes in multiple steps of a pathway or signal cascade. It is also beneficial if it can also take care of related or unrelated co-morbidities.

Chaudhari et al. (2017) have pointed out that polypharmacology can also help meet the challenge of drug resistance as exemplified in a drug molecule binding

to several protein kinases, including mutants, which are formed as a part of the development of drug resistance (Chaudhari et al. 2017). This has been particularly helpful in the context of some cancers. They also point out that "Increasing evidence acquired from genomics studies have shown that many biological systems are insensitive to a single attacker.... Interestingly, it is also found that over 40% of these targets are involved in more than one disease through molecular network analysis" (Chaudhari et al. 2017). The authors have also discussed why a single drug hitting multiple targets is better than the use of combination therapy. A significant advantage is that the pharmacokinetic profile is more predictable. Also, the risk of toxicity/side effects is lower if a single drug is being used. This study is also a good source of a fairly comprehensive list of methods and algorithms used in polypharmacological studies (Chaudhari et al. 2017).

Broadly, the approaches can be classified into ligand-based methods, structure-based methods, network-based methods, and bioinformatics methods. Ligand-based methods rely upon the assumption that ligands similar in structure and/or shape will bind to similar targets. Structure-based methods screen libraries of potential drug molecules for binding to the desired set of targets. Both high throughput screening methods and docking are used for such approaches. Network-based methods use genomic, proteomic, and metabolomic databases to identify the desired protein-protein or protein-ligand interactions. Bioinformatics exploits gene expression data (Chaudhari et al. 2017).

In order to come back to the theme of antibiotic resistance, antibiotics are used in many diseases which are not caused by bacterial infections. In some cases, it is to prevent or control secondary infections. There are other cases that are examples of repurposing of antibiotics (Song et al. 2016; Alsalahat et al. 2021; Khan et al. 2021). Talat et al. (2022) discussed the wisdom of repurposing antibiotics in view of the serious concerns about the escalating spread of antibiotic resistance (Talat et al. 2022). The authors suggested that repurposing of non-antibiotic drugs, wherever possible, should be preferred and cite two examples wherein non-antibiotic options have been used in the treatment of cancer and Alzheimer's (Timar et al. 2005; Advani and Kumar 2021). They were especially concerned that in many instances of antibiotic repurposing, last-resort antibiotics are used, as the pathogens have already become resistant to other antibiotics. Even worse, the case has also been mentioned where the use of deoxycycline resulted in the development of co-resistance to one such last-resort antibiotic, Colistin (Narendrakumar et al. 2020). Many cases of repurposed antibiotics in cancer or Alzheimer's involve their use for a prolonged period at fairly large doses. This increases the likelihood of the development of antibiotic resistance.

Gupta et al. (2020) have discussed parameters that lead to drug promiscuity (Gupta et al. 2020). Molecular weight/size, hydrophobicity, and conformational flexibility of the drug molecule do not necessarily correlate highly with its promiscuity. The similarity in binding sites of the targets is a much better indicator for a drug to bind to such targets (Gupta et al. 2020). They cite an earlier publication, in which out of 164 drugs examined, about 71% had at least two targets, and the targets had similar binding sites. They also cite the examples of Hsp27 (target in cancer cells) and thymidine kinase (target in herpes virus), which bind to Brivudin (which led to this drug for herpes being repurposed for pancreatic cancer). Both

target proteins have merely five different amino acids at similar positions. Highly promiscuous drugs acarbase (originally used for diabetes), methotrexate (originally used for cancer), and niacinamide (originally a drug for some skin diseases) have been found to bind to >15 targets; the sequence similarity does not exceed 18% in the case of their promiscuous targets (Gupta et al. 2020).

However, it is important to note that while the sequence similarity in targets is a good indication, its absence does not necessarily preclude the same drug binding to such targets. Meyers et al. (2018) cited an example where screening a small library of privileged structures (please see later discussion on their definition) led to a drug binding to two receptors, which belonged to different families (Meyers et al. 2018). The authors also provided a useful discussion on ways of discovering pharmacological drugs. The first kind is probes, in which two structures (with the potential to bind to different targets) are linked with a flexible linker. The design is similar to the bioorthogonal probes often used in chemiproteomics (Binoy et al. 2023). PROTACS (Proteolysis Targeting Chimeras, which are bifunctional molecules with two heads connected by a linker that can selectively degrade harmful proteins in cells) is also included in this type. The second type of drug binds to targets unrelated to well-established targets and is toxic in high concentrations. The third type, of course, is those with sequence similarity among targets. Meyers et al. (2018) mention high sequence similarity, but as we have discussed above, often limited similarity in binding pockets may be good enough. The fourth type is actually the focus of their work and refers to high-affinity interactions of the ligand with protein targets belonging to different families. Here, a bisamide showed binding to both pirin (a member of the cupin superfamily) and a protein kinase B-Raf. Figure 3 represents the crystal structures of pirin and B-Raf and illustrates a complete lack of structural similarity between these proteins. Interestingly enough, the binding pockets of these

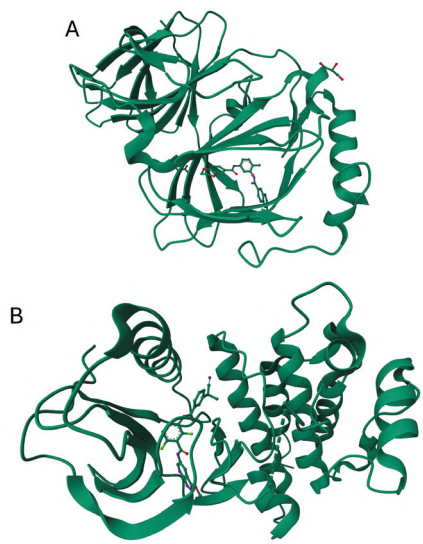

Figure 3. X-ray crystal structures of pirin (PDB ID: 6H1I; A) and B-Raf (PDB ID: 4G9C; B).

two dissimilar proteins showed no similarity as well. This observation is a powerful example of the inapplicability of the lock and key hypothesis (Meyers et al. 2018).

The role of intrinsically disordered regions in binding to small molecular weight entities and other proteins is fairly well-known (Gupta and Uversky 2023a; Gupta and Uversky 2023c; Gupta and Uversky 2023d; Gupta and Uversky 2023e; Gupta and Uversky 2024). However, "short foldable sequences rather than the low complexity, intrinsically disordered regions," which are part of the interfaces in protein-protein interactions, have also been exploited for identifying drug molecules. These are yet to be exploited sufficiently for polypharmacology.

Privileged Scaffolds and Search for the Polypharmacological Drugs

Choo and Chai (2023) have recently reviewed examples of natural products that have been shown to be useful in polypharmacology. The authors cite an earlier computational study by Fang et al. (2018), who showed that several natural products approved by the FDA (such as resveratrol, quercetin, genistein, and fisetin) possessed anti-cancer properties for several types and subtypes of cancer (Fang et al. 2018). They make an insightful observation that "Produced by biosynthetic pathways that require successive binding to various enzymes, natural product scaffolds have evolved to interact with multiple proteins, giving rise to a range of biological activities" (Choo and Chai 2023). Hence, their structures are potentially well-suited to bind to multiple targets/signaling cascades. A striking example given by them is that of andrographolide, an active constituent of the plant *Andrographis particulata*, which has been used in traditional medicine for treatments of "fever, diabetes, respiratory infections, malaria, and hypertension."

The interdependence of drug promiscuity and protein promiscuity is also highlighted by the concept of privileged scaffolds. The concept was originally proposed by Evans et al. (1988), who observed that most small molecular-weight drugs have a rather narrow range of structural motifs/scaffolds (Evans et al. 1988). This results in similarity in their binding sites as well. This would result in a significant level of "cross-binding" (Evans et al. 1988). Gupta and Roy (2021) have provided many examples of privileged scaffolds, structures of drugs wherein these scaffolds are present, and their corresponding therapeutic applications. They also point out these are useful in envisaging drug repurposing potentials (Gupta and Roy 2021). For example, Qin et al. (2020) have discussed the use of chalcone hybrids as privileged scaffolds in drug discovery programs for antimalarials (Qin et al. 2020).

This approach is related to scaffold hopping (also known as lead hopping, which is a drug discovery strategy that involves modifying the core structure of a molecule to identify similar active compounds with different molecular backbones), which is a subset of the broader approach of bioisosteric replacement (Brown et al. 2018). A bioisostere is one where the structure of the parent molecule is modified to optimize desirable properties while (at least) not diminishing the essential biological property. Generally, replacement is done using functionally equivalent substituents. In scaffold hopping, the core scaffold is changed in such a way that it again retains the capability

of binding to the target but creates opportunities for altering substituents (Brown et al. 2018).

Another important method for discovering drugs with polypharmacological potential is the fragment-based drug discovery approach (Bottegoni et al. 2012). This leads to a stepwise addition of the fragments to a single compound (scaffold molecule) to enhance its efficacy. In general, there is enough evidence to support the notion that "fragment-based approaches are likely to detect promiscuous molecules" (Bottegoni et al. 2012). The potential fragments are identified by weak binding to the target receptor(s). Surface plasmon resonance (SPR), isothermal titration calorimetry (ITC), NMR, and X-ray crystallography have proved useful in estimating these weak bindings. The authors also outlined how multiple solvent X-ray structure determination can prove useful for this (Bottegoni et al. 2012). Here, the target protein solvated with different organic solvents can be looked at. The small molecules that bind to the protein are potential scaffolds to add fragments/substituents to attain a useful drug structure (Bottegoni et al. 2012).

Polypharmacology vs. Drug Repurposing

Polypharmacology involves a drug molecule that is capable of binding to several targets. These may include some "off-targets" (non-intended or unanticipated targets). Drug repurposing is about exploiting the bindings to these "off-targets." Some authors view polypharmacology and drug repurposing as terms with identical meanings. For example, "Targeting multiple targets with a single drug by employing drug repurposing known as the polypharmacology approach may be an optimized strategy for developing effective therapeutics" (Jamir et al. 2022). However, there is a distinction. In polypharmacology, the aim is to use a single drug to hit more than one target, which may relate to a complex disease involving multiple steps of a pathway/cascade or these multiple targets related to different diseases. In drug repurposing, the intention is to use a drug that already has regulatory approval for a single specific disease (or many diseases) for yet another disease. A drug that is being repurposed has to be promiscuous. In that sense, we can say that it has to be polypharmacological. Both approaches rely upon drug promiscuity. In order to stick close to the analogy, the magic bullet in such cases is replaced by a magic shotgun. The magic part is that in both polypharmacology and drug repurposing, one still wants to minimize the side effects (the scatter of the shotgun, which can cause collateral damage) (Kabir and Muth 2022).

Conclusion and Future Perspectives

Both promiscuity of the target protein and the drug promiscuity ultimately center on the lack of specificity proteins display. While the classical enzymology developed by exploiting the virtue of protein specificity, the last few decades have turned the tide. Increasingly, biologists are exploiting promiscuity (lack of specificity) for a variety of applications. It is now largely accepted that promiscuity is an inherent property of proteins and is one of the ways by which proteins evolve. Thus, there is an umbilical cord between specificity and promiscuity. If we have to learn how to repurpose drugs

in a rational way, understanding promiscuity also requires a better understanding of the nature of drug-drug-receptor interactions (Gupta and Uversky 2024).

Contrary to the general perception, we still do not completely understand the molecular details of the twin magic of high specificity and high turnover rates displayed by some enzymes (or, for that matter, other kinds of proteins with biological activities). The recent work of Karvelis et al. (2024) shows that it is not just conformations associated with the ground state or the transition state that are important; conformational dynamics of the enzyme-substrate complex even before reaching the transition state represent an interesting target for manipulating the protein specificity (Karvelis et al. 2024). The authors report "…enzyme mutants with a significant improvement in calculated specific activity relative to wild type (WT), with the fastest variant's increase in calculated k_{cat} being (2±1) × 10^4-fold. Collectively, these results suggest that introducing mutations designed to increase the population of reaction-promoting conformations of the enzyme-substrate complex before it reaches the barrier can provide an effective approach to engineering improved enzyme catalysts" (Karvelis et al. 2024). The fact that the eight mutants of the isomerase had altered specificity shows that the populations provide a wide window of opportunity in altering specificity (Karvelis et al. 2024). Could this approach provide a way of achieving targeted promiscuity for polypharmacology or drug repurposing?

Similarly, Zhang's group (2024) underlined how the engineering of loops can impact the active site functionality (Xi et al. 2024). That, of course, leads to another important aspect of protein structure (or lack of it!). Protein loops are often disordered (Feller and Lewitzky 2012; Mughal and Caetano-Anolles 2023). Intrinsic disorder is often the basis of protein promiscuity and is beginning to be exploited in drug discovery programs. Rather than basing our drug repurposing strategies on either the structure or the disorder of the target protein, we may profit from readjusting our focus to follow a more holistic approach by looking at protein function through the lens of the continuum model, in which the conformational dynamics includes protein flexibility as well as the intrinsic disorder (Gupta and Uversky 2024).

To sum up, further strides into polypharmacology and drug repurposing would be made by studying drug promiscuity along with protein promiscuity and protein moonlighting. Another interesting dimension of drug repurposing is discussed by Chaganti et al. (2024), who deliberated on the repurposing of drugs for other species (Chaganti et al. 2024). As a part of the conservation efforts toward saving endangered species, 5-Fluorouracil was used to treat cancer in a green sea turtle. This repurposing was described as "conservation medicine" (Chaganti et al. 2024).

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CHAPTER

2

Approaches to Identify and Repurpose Drugs for Neurodegenerative Diseases

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Introduction

A never-ending hunt has been conducted to find new medications in a variety of therapeutic areas. Repurposing, sometimes known as drug repositioning, has recently attracted attention. The novel strategy of drug repurposing (DR) has several benefits over the traditional drug discovery method and has created new opportunities in the fields of medicinal chemistry and pharmacology (Kakoti et al. 2022). Notable examples of successfully repurposed drugs include aspirin, thalidomide, sildenafil, and dimethyl fumarate. The approach of DR can be divided into three main categories: drug-centric, disease-centric, and target-centric. Disease pathology, omics data, scientific literature, and drug compound databases are important sources of information for DR. A new drug's development usually takes 10 to 15 years and costs about $2.6 billion. Repurposing drugs has the potential to cure diseases that are currently incurable while also saving a substantial amount of time and money.

Neurodegenerative diseases (ND), which affect the peripheral nervous system (PNS), are a major focus of DR efforts. Alzheimer's and Parkinson's diseases are the most common NDs (Lamptey et al. 2022). With the global population increasing, more diseases are being categorized under NDs. Although some medications are available for treating NDs, they primarily slow the progression of the diseases rather than cure them. Consequently, pharmaceutical companies are increasingly turning to DR to find effective treatments for NDs. This review discusses the historical and

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contemporary instances of DR at different phases of the drug development process, with a particular emphasis on NDs and the associated risks and concerns, such as the scientific, regulatory, and commercial aspects (Zhang et al. 2016).

Strategies for DR

According to the U.S. Census Bureau, 8.07 billion people were predicted to live on Earth as of September 2, 2024 (United States Census Bureau, 2024). This indicates a 0.91% growth rate or an increase of 74 million people from 2023 (Macrotrends 2024). In addition, the number of elderly individuals has increased, which is contributing to the rise of the global population. Every nation's population is experiencing an increase in the number and percentage of senior people. By 2030, it is projected that 1 in 6 persons on the planet will be 60 years of age or older. Over 80 years old, life expectancy is gradually rising in wealthy nations. As a nation's population does tend to be older on average, the occurrence and progression of ND have become more common. According to Hou and their team, the primary risk factor for almost all ND, including Alzheimer's disease (AD) and Parkinson's disease (PD), is aging. Since AD alone can affect between one-third and half of persons over the age of 85, the number of people affected by the disease is expected to rise to 135 million by 2050 (Hou et al. 2019). Without effective treatments to slow the spread of these diseases, NDs are predicted to have catastrophic effects on people, families, and society as a whole (Kakoti et al. 2022). The entire drug development process, from target selection and validation to authorized medication usage, is a challenging procedure that takes time to complete (Durães et al. 2018). DR is a popular approach nowadays that addresses several issues with the de novo creation of completely new pharmaceuticals. It lowers failure rates during the clinical development and testing stages, expedites the discovery process, and is effective, affordable, and relatively risk-free (Tanoli et al. 2021).

Nevertheless, identifying new drug-disease associations is the primary challenge in drug repositioning. There are two primary DR techniques, on-target and off-target, as well as a variety of approaches to address this problem (Rudrapal et al. 2020). The previously recognized pharmacological mechanism of a drug molecule is connected to a novel therapeutic implication in on-target (target-centric) drug delivery. The disease is different in this plan of action, but the therapeutic candidate's biological target remains unchanged. It combines hybrid techniques, computational methodologies, and biology-based experimental procedures (Ferreira and Andricopulo 2016). Conversely, a drug candidate's pharmacological mechanism goes unnoticed in the off-target (drug-centric) profile. Pharmaceuticals and drug candidates react to novel targets that fall beyond their initial purview and have novel curative uses. As a result, the indications and goals are unique (Ashburn and Thor 2004). Three important phases are highlighted in drug discovery: the generation of candidate molecules, preclinical analysis, and clinical trial. Finding pertinent medications with prospective therapeutic indications is crucial for synthesizing candidate medicines. Significant progress has been made in our understanding of the biology of NDs. Similarly, a wealth of new easily available information has made drug reprofiling a more straightforward approach to drug discovery. Abundant data

from clinical, mechanistic, and epidemiological studies, the creation of biomarkers, and several well-validated models based on both cell and animal models are all incorporated into these. These days, the most common drug reprofiling methods among NDs are based mostly on preclinical modifications in rodent models and ad hoc clinical and epidemiological risk assessment in human trials (Ashburn and Thor 2004). However, more exact and standardized techniques for both activity-based and computational methodologies should be implemented to achieve superior DR in NDs. Future efforts in ND drug repositioning will be greatly accelerated and encouraged by the availability of contrasting sophisticated tools in genomics and bioinformatics, as well as by the rapid advancement in the scientific study of disease. Two alternative and complementary approaches, activity-based/experiment-based phenotypic screening and theoretical/*in-silico*-based/computational approaches, may be widely used to explore novel therapeutic liabilities for neurodegenerative disease (Rudrapal et al. 2020). It is also possible to approach DR by combining the two disciplines.

Experiment-based Approach

This approach involves identifying original compounds for new pharmacological uses through experimental assays, combining protein target-based and cell or organism-based screens *in vitro* and/or *in vivo* disease models. Although the activity-based approach is both time-consuming and labor-intensive, it has the advantage of generating fewer false positive hits during the screening process. Experimental drug repositioning involves various strategies, including cell assays, target screening, animal models, and clinical approaches (Lionta et al. 2014; Oprea and Overington 2015).

Two commonly used proteomic techniques for analyzing drug candidates are affinity chromatography and mass spectrometry (Brehmer et al. 2005). Today, drug target analysis is closely linked with drug repositioning, which differs from drug discovery in terms of modifying drug targets. The binding affinity of drug ligands can be predicted using a cellular thermal stability assay, which maps the interaction patterns of intracellular targets. This method has uncovered numerous on- and off-targets for various clinically approved drugs. New biological targets for well-known drugs are often discovered through affinity matrices, particularly in the field of kinases (Kakoti et al. 2022).

Phenotyping Screening

Phenotypic screening encompasses the methods implemented to characterize drugs' beneficial or adverse effects in association with a disease. Myriad libraries and databases of chemical drugs could be screened in one stroke. This technique uses two broad strategies based on cell-based screens [high throughput screening (HTS)] or utilizing whole organisms. Several cell lines are now available for work on cell-based assays including human or animal cell lines, induced pluripotent cell lines (iPSCs), transformed cell lines, cancer cell lines, etc. Cell signaling pathways assay, cell viability assay, and disease mechanistic assays are notable cell-based assays frequently employed for DR (Iljin et al. 2009; Vincent et al. 2022).

In-silico Approaches

The *in-silico* approach to DR leverages computational tools to identify new therapeutic uses for existing FDA-approved drugs, particularly for NDs. This strategy involves the virtual screening vast chemical and drug libraries using molecular docking simulations to predict drug-target interactions (Kakoti et al. 2022). By analyzing how well a drug binds to its target and the kinetics of this binding, researchers can assess the potential efficacy of a drug for a different disease. Additionally, *in-silico* methods integrate various data sources, including genomic, proteomic, and transcriptomic information, to identify drugs that can modulate disease-related gene expression patterns. Despite being time and cost-efficient, this approach often produces false positives, making it essential to combine these findings with other data types and experimental validation (Alam et al. 2010). Nonetheless, the computational DR strategy is particularly valuable for its ability to rapidly and systematically identify potential new treatments, especially when addressing the challenges of ensuring drug penetration across the blood-brain barrier in neurodegenerative disorders (Kakoti et al. 2022).

Artificial Intelligence (AI) and Machine Learning (ML)

Several companies in recent years have developed and refined artificial intelligence (AI) and machine learning (ML) based drug discovery frameworks. These techniques are good at connecting different types of data. The development of ML methods to effectively search for transcriptional, structural, and clinical information has gained much attention (Kakoti et al. 2022).

ML is one type of artificial intelligence. Applying statistical techniques to uncover previously unknown associations and patterns in the data does make intensive interrogation of multiple datasets easier. Recently, the approaches have shown promise when repurposing drugs for NDs. ML algorithms have three main categories: supervised, unsupervised, and reinforcement learning approaches. The supervised ML algorithms are the current techniques most often used with data relevant to NDs. IBM Watson developed a semantic model of ALS-associated RNA-binding proteins using AI-based text-mining techniques, which could serve as model therapeutic targets. By applying this approach to a novel collection of RNA-binding proteins, IBM Watson may be able to identify putative RNA-binding linked with ALS (Bakkar et al. 2018).

A unique computational method based on data integration and machine learning was described in a study to forecast medication repositioning. The study's methodology depended on continuously analyzing categorization discrepancies as legitimate prospects for reclassification. The results were highly definitive and consistent with multiple studies from the literature (Napolitano et al. 2013). Another investigation introduced a novel approach called "PREDICT," which was predicated on the idea that similar medications may also be recommended for similar diseases (Gottlieb et al. 2011). The approach predicted drug candidates with extremely high specificity and sensitivity, making it more appealing than the current approaches for forecasting large-scale pharmacological indications for newly licensed medications and unproven compounds.

Deep learning (DL) methods are one example of a revolutionary computational technology that has shown remarkable promise in recent years to amplify the capabilities of DR methods. DL uses techniques referred to as deep neural networks (DNNs), which include artificial neural networks with a large number of hidden layers between the input and output layers (Ma et al. 2015). A study that focused on deep learning was conducted by Aliper and his team, wherein the scientists verified that deep neural networks (DNNs) trained on large transcriptional response datasets could classify various medications into therapeutic groups based only on gene transcription profiles (Aliper et al. 2016). Positive results were also attained through the use of a deep learning-based algorithmic framework known as DeepDTIs (Drug Target Interaction), which determined drug-target interactions by utilizing existing interactions and chemical structures (Wen et al. 2017).

Genome-Wide Association Studies (GWAS)

Genome-wide association studies (GWAS) provide valuable insights into the genetic basis of complex diseases, offering promising avenues for DR (Kakoti et al. 2022). By identifying genetic variants associated with disease, GWAS can pinpoint specific genes and biological pathways that are implicated in disease processes. This information can be leveraged to discover existing drugs that may target these genetic pathways or modify the activity of associated genes. Approaches such as linking genetic loci to pharmacologically modifiable pathways, conducting transcriptome-wide association studies, and performing gene-set association analyses can reveal potential drug targets and repurposing opportunities. Additionally, techniques like Mendelian randomization, which infers causality from genetic data, and polygenic scoring, which assesses the cumulative effect of multiple genetic variants, can further guide the identification of drugs that might be effective for new indications. Overall, integrating GWAS findings with DR strategies offers a promising method to expedite the discovery of new treatments by harnessing existing therapeutic agents (Reay and Cairns 2021). A clinical candidate called Biib-033 (Biogen Idec, Cambridge, MA, USA) is an example of how GWAS is being used in DR. It is an antibody that targets the leucine-rich repeat and immunoglobulin domain-containing 1 (LINGO-1), and it has been developed for multiple sclerosis. It has been suggested that LINGO-1 may be repurposed for treating vital tremor diseases after two GWAS studies identified it as a target for the neurological condition essential tremor (Clark et al. 2010; Stefansson et al. 2009).

Network-based Method

Convolutional Neural Networks (CNNs) offer a powerful approach to DR by analyzing complex patterns in diverse datasets. By processing chemical structures of drugs, represented as SMILES strings, CNNs can uncover patterns and similarities that suggest new therapeutic uses for existing drugs. They are also adept at integrating data on drug targets, pathways, and enzyme interactions to predict potential new applications. CNNs excel in handling multi-modal data, combining chemical substructures with target and pathway information, providing more accurate predictions of drug interactions and repurposing opportunities. Their ability to achieve

high accuracy, as demonstrated in predicting drug-to-drug interactions, highlights their effectiveness in identifying novel uses for existing drugs. Additionally, CNNs benefit from iterative refinement through experimentation with hyperparameters and drug features, enhancing their predictive performance and making them a valuable tool in the quest for new therapeutic indications (Asfand-e-yar et al. 2024). Although the application of CNNs in DR has not yet been thoroughly used, from its application, we can consider it a powerful approach in DR to treat different NDs.

Current Therapeutic Approaches to Treat ND By Using DG

NDs are conditions that impact both the central and peripheral nervous systems. There are more than 600 different types of neuropathological diseases, such as epilepsy, brain tumors, Parkinson's disease, and stroke. In light of the expanding worldwide population, there are currently more NDs than ever before (Kumar et al. 2022; Matilla-Dueñas et al. 2017; Siuly and Zhang 2016). According to the World Health Organization, NDs affecting motor function will surpass cardiovascular disease as the second leading cause of death within the next 20 years. Because of its poorly understood molecular basis, no ND is currently curative, and the available medications only manage symptoms or slow disease progression (Onyango et al. 2021).

DR is the best new strategy for developing an effective treatment for NDs because the pharmacokinetic and pharmacodynamic features of the drug are previously understood. Numerous studies have examined the potential of older drugs for the most significant NDs, including multiple sclerosis (MS), Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), and amyotrophic lateral sclerosis (ALS) (Durães et al. 2018). A list of medications that have been repurposed for certain NDs is shown in Figure 1.

Alzheimer's Disease (AD)

AD causes 80 percent of cases of dementia in older adults. Its symptoms include progressive memory loss, an inability to learn, and a decline in behavior and function. While the precise pathophysiology of AD remains unclear, amyloid-β plaque accumulation in the brain is believed to be associated with the disease, leading to the eventual degeneration of neurons and synapses (Scheltens et al. 2016). According to Appleby and his team, the majority of AD drugs are used to treat cognitive deficits or other symptoms, and they are most effective when used early. Drugs such as galantamine, donepezil, rivastigmine, and other cholinesterase inhibitors are frequently used for AD (Appleby et al. 2013). Here, we have mentioned different therapeutic approaches used to treat AD, such as the amyloid-directed antibody approach, cholinesterase inhibitors, glutamate regulators, and repurposed drugs for AD.

Amyloid-directed Antibody Approach to Treat AD

Anti-amyloid monoclonal antibodies, which reverse the amyloid plaque formation

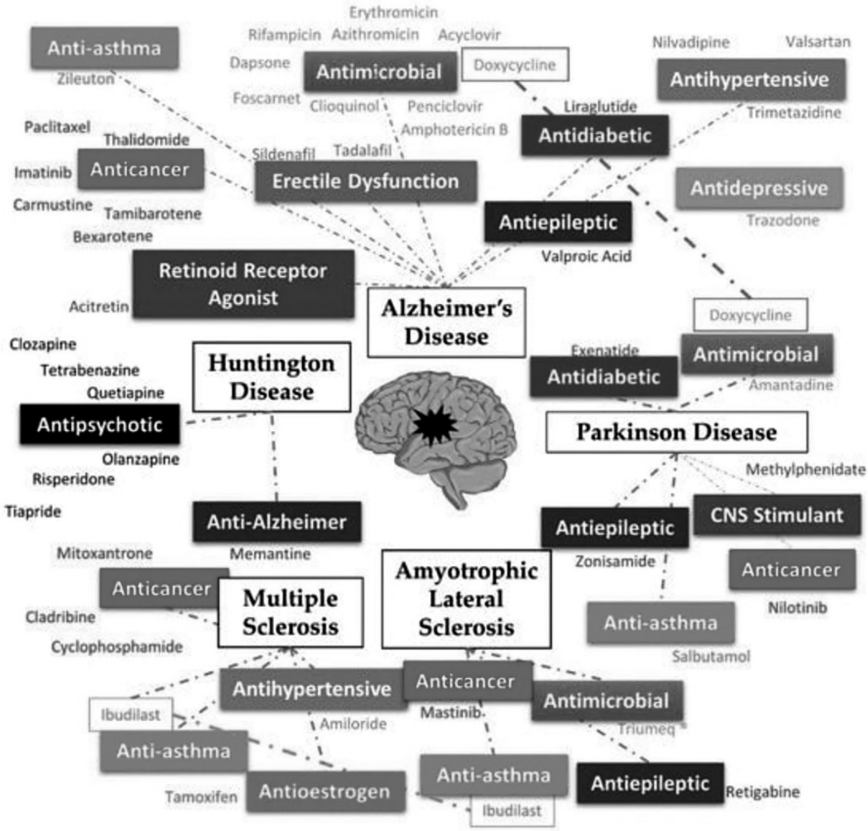

Figure 1. Some examples of repurposed drugs used in NDs, adapted from Durães et al. (2018) via CC by 4.0 license. ⏎

and pathology, represent the first class of disease-modifying therapies for AD that effectively slow clinical decline by targeting the underlying biological processes of the disease. In earlier days, these anti-amyloid monoclonal antibodies were often used in passive immunization to treat AD (Van Dyck 2018). These groundbreaking agents have the potential to delay the progression of AD into more severe stages of cognitive impairment. The success of clinical trials for anti-amyloid monoclonal antibodies lends strong support to the amyloid hypothesis and reinforces the validity of amyloid as a target for AD drug development. This achievement results from a persistent and rigorous application of neuroscience, addressing some of the most significant challenges in modern medicine (Cummings et al. 2023; Kakoti et al. 2022). The advent of these transformative therapies is expected to catalyze the further development of additional anti-amyloid monoclonal antibodies and other anti-amyloid therapies, as well as treatments targeting different aspects of AD pathology, and the development of novel therapeutic strategies for a range of NDs. However, monoclonal antibodies are not without side effects. During the initial

treatment phase, patients must be carefully monitored for amyloid-related imaging abnormalities (ARIA) and potential infusion reactions (Cummings et al. 2023).

Introducing these agents marks a significant milestone in developing disease-modifying therapies for AD, setting the stage for future therapeutic advancements that may offer reduced ARIA risk, more convenient administration methods, and enhanced efficacy. The novel nature of these treatments introduces new challenges and responsibilities for patients, caregivers, healthcare providers, insurers, and healthcare systems. Effective collaboration among all stakeholders is crucial to fully realize the therapeutic potential of these agents and to ensure their widespread availability (Cummings et al. 2023). Aducanumab (Aduhelm) is the first disease-modifying drug approved for patients with Alzheimer's disease, receiving approval in June 2021. The drug is delivered through an intravenous (IV) infusion, which takes about an hour and is administered every four weeks. Aducanumab is an IgG1 monoclonal antibody that specifically targets extracellular Aβ plaques in the brain, aiding their clearance (Lamptey et al. 2022).

Cholinesterase Inhibitors (ChEIs) to Treat AD

Acetylcholine (ACh) is one of the primary neurotransmitters in the brain. In AD, cholinergic deficits are a hallmark of disease progression, contributing to cognitive decline and dysfunction. Acetylcholinesterase (AChE) is a crucial target for improving symptoms of AD, as these cholinergic deficits are both early and consistent findings in the disease (Kakoti et al. 2022). The success of AChE inhibition in treating myasthenia gravis demonstrated the potential of this approach as a therapeutic target. This success paved the way for the development and approval of drugs such as tacrine, donepezil, rivastigmine, and galantamine for managing AD symptoms. Since then, the development of additional cholinesterase inhibitors (ChEIs) has continued (Kabir et al. 2021). Research has been done on galantamine, an alkaloid present in Galanthus species, as a possible therapy for myopathies and peripheral neuropathies. It could potentially block muscle acetylcholinesterase. Galantamine helps reverse neuromuscular blockage during anesthesia due to its capacity to enhance nerve impulse transmission. The majority of galantamine use in the 1960s and 1980s was restricted to Italy, Bulgaria, Germany, and France, where it was marketed under the name Nickel®. Galantamine was not added to the list of medications used to treat AD until 2000, even though its potential benefits for AD were initially studied in the 1980s (Mucke 2015).

Glutamate Regulators to Treat AD

Glutamate is the primary excitatory neurotransmitter in the brain, but its excessive activation, particularly through NMDA receptors on postsynaptic neurons, can cause neuronal damage and contribute to neurodegeneration. While completely blocking NMDA receptors leads to severe side effects, the development of memantine, an uncompetitive NMDA receptor antagonist, offered a solution. Memantine allows for beneficial NMDA receptor activity while protecting against the harmful effects of overactivation. Memantine, approved in 2003 for treating moderate-to-severe AD, is administered at a dose of 5–20 mg per day. Clinical studies have shown that

memantine alone improves cognitive function in AD patients compared to a placebo. When combined with acetylcholinesterase inhibitors, its efficacy is enhanced for up to a year compared to using memantine alone. However, this class of drugs primarily addresses symptoms rather than the underlying pathology of AD (Lamptey et al. 2022).

Repurposed Drug for AD

Depending on the organism, pathogens can enter the Central Nervous system (CNS) by several different routes, which could hasten the onset of AD. Pathogens first attach and damage the blood-brain barrier (BBB) (Orgogozo et al. 2003). Certain viruses, such as the herpes virus, can become latent following the initial infection and then reactivate decades later in older individuals, leading to potentially fatal symptoms that are delayed (Goddard 2005). A 2020 study model states that Aβ plaque and neurofibrillary tangles (NFTs) were produced in the olfactory cortex and hippocampal regions of immunocompromised individuals who were exposed to *C. pneumoniae* through their noses (Sundar et al. 2020). Accordingly, research has been done to determine whether antimicrobials such as Rifampicin, Amphotericin B, acyclovir, penciclovir, foscarnet, etc., may be utilized to treat AD, particularly its symptoms (Iqbal et al. 2020).

Since type 2 diabetes has been linked to AD, antidiabetics are also utilized to treat the condition. Studies show that the brains of AD patients are no longer as receptive to insulin signaling. Insulin therapy has been demonstrated to protect the brain from injury and regulate the levels of phosphorylated tau protein, all while improving memory and cognition. Furthermore, insulin can stimulate brain stem cell activation, proliferation, and repair. Therefore, drugs that influence insulin release may be advantageous for AD. For example, in addition to increasing insulin production, analogues of glucagon-like peptide 1 (GLP-1) may also decrease tau phosphorylation, amyloid-β plaque formation, neuronal cell death, and increased neuronal function, in AD (Perry et al. 2003; Zhao et al. 2004). By using the concept of drugs repurposing different anticancer, antiepileptic, antidepressant, antiasthmatic, etc., drugs are also used to treat AD. A list of repurposed medications used for AD is shown in Table 1.

Parkinson's Disease (PD)

Parkinson's disease (PD) is a complex neurological illness that makes it difficult for a patient to walk. PD primarily affects dopamine neurons in the putamen and caudate regions of the brain. The activities of mitochondria are reduced in the substantia nigra of Parkinsonian brains due to dopamine inhibition, higher ROS and RNS generation, and mitochondrial DNA deletion (Reeve et al. 2018; Ryan et al. 2015). In PD, dopamine levels decrease due to oxidation by both monoamine oxidase (MAO) A and B. PD is characterized by a primary tremor, akinesia, stiffness, bradykinesia, postural instability, and subsequent motor symptoms such as freezing of gait, micrographia, and speech problems. Non-motor symptoms of PD can include autonomic dysregulation, sensory impairment, neurobehavioral abnormalities, and sleep disturbances (Kakoti et al. 2022).

Table 1. Some examples of repurposed drugs for AD ⏎

Drug	Repurposed	Earlier Used	References
Rifampicin	Due to a decreasing formation and an increasing clearance of amyloid-β, it has demonstrated a dose-dependent reduction in amyloid-β fibrils.	Mycobacterium infection	(Tomiyama et al. 1996)
Bexarotene	It is successful in reversing neurodegeneration, improving cognition, and reducing amyloid-β levels in mice overexpressing familial AD mutations.	Cutaneous T-cell lymphomas	(Tousi 2015)
Valproic acid	It is suggested as a neuroprotective treatment for AD because of its capacity to reduce the formation of amyloid-β plaques in transgenic mice and ameliorate memory deficits.	Antiepileptic drug	(Smith et al. 2010)
Carmustine	At a non-toxic dosage, carmustine significantly inhibits the formation of amyloid-β in cells that overexpresses the precursor protein of the amyloid protein.	Alkylating agent in brain cancer	(Hayes et al. 2013)
Acyclovir, foscarnet, penciclovir	Reduces amyloid-β and phosphorylated tau protein in AD cell cultures	Antiviral drug	(Wozniak and Itzhaki 2010)
Tamibarotene	It can impact multiple mechanisms implicated in the pathophysiology of AD, such as those governing brain cell production of pro-inflammatory chemokines and cytokines, senescence-affected animal behavior, and cortical acetylcholine levels.	Acute promyelocytic leukemia	(Fukasawa et al. 2012)
Zileuton	Zileuton can provide therapeutic effects for AD because it suppresses 5-lipoxygenase. This is because 5-lipoxygenase is an intriguing target in this context, and it has been found to be more abundant in AD. Studies employing zileuton in mice shown a reduction in the buildup of amyloid-β.	Antiasthma drug	(Meco et al. 2014)

Trazodone	Trazodone has shown promise in reducing signaling through the PERK/eIF2α-P branch of the unfolded protein response, which is hyperactive in AD patients and damages cells' ability to regulate translation.	Antidepressant drug	(Halliday et al. 2017)
Paclitaxel	Paclitaxel only reaches a tiny section of the CNS and can be a substrate for P-gp, but because it decreases tau protein phosphorylation, it is instrumental in treating tauopathies.	Antimitotic drugs used in lung cancer, breast, and ovarian cancer	(Brunden et al. 2011)
Valsartan	Chronic unfavorable stress is one of the primary environmental variables associated with AD, as it can raise brain levels of angiotensin II. Angiotensin II elevations have been demonstrated to be associated with amyloid genesis; hence, postponing the loss of cognitive processing may be possible using angiotensin receptor blockers. Valsartan also promotes the release of acetylcholine while lowering inflammation, vasoconstriction, and mitochondrial dysfunction.	Antihypertensive drug	(Culman et al. 2002)
Thalidomide	It can lessen the death of hippocampal neurons by inhibiting tumor necrosis factor-α.	It stops the proliferation of endothelial cell growth, angiogenesis, and disruption of the blood-brain barrier.	(Ryu and McLarnon 2008)
Acitretin	It is identified to increase the modulation of antioxidants and enzymes that remove amyloid-β.	Retinoid receptor activator	(Tippmann et al. 2009)
Trimetazidine	It can effectively myelinate both healthy and injured axons, cross the blood-brain barrier, reduce the creation of free radicals, and improve axonal regeneration.	Anti-ischemic drug	(Hassanzadeh et al. 2015)
Tadalafil/Sildenafil	Sildenafil demonstrated efficacy in mitigating amyloid-β and lowering neuroinflammation in elderly animal models. Moreover, Tadalafil demonstrated neuroprotection and improved cognitive function.	Erectile dysfunction drug	(García-Barroso et al.; Zhang et al. 2013)

(*Contd*.)

(*Contd*.)

Drug	Repurposed	Earlier Used	References
Erythromycin, Azithromycin	By stopping the synthesis of the amyloid precursor protein, they reduce the amount of amyloid-β in the brain.	Macrolide antibiotics	(Appleby et al. 2013)
Amphotericin B	It has been shown to decrease the synthesis of amyloid-β; however, it shows toxicity.	Antifungal drug	(Hartsel and Weiland 2003)
Tetracyclines	Researchers have found that it suppresses the production of amyloid-β and promotes the breakdown of fibrils.	Antibiotic	(Diomede et al. 2010)
Ghrelin	Ghrelin and its deacylated precursor have been demonstrated to have neuroprotective benefits by lowering the rise of interleukins from amyloid-β and preventing programmed cell death.	Peptide hormone controls appetite and is synthesized in the alimentary tract	(Wagner et al., 2017)
Clioquinol	It shows a decrease in amyloid-β plaques in the brains of transgenic mice.	Antiparasitic, antifungal drug	(Grossi et al. 2009)
Liraglutide	It shows cerebral penetration and physiological alterations in the brain that enhances learning and decreases the growth of amyloid-β and inflammation.	Antidiabetic drug	(McClean et al. 2011)

Levodopa, carbidopa, amantadine, rotigotine, dopamine agonists, catechol-O-methyltransferase (COMT) inhibitors, anticholinergics, selegiline, rasagiline, safinamide, etc., are among the medications used to treat PD (Gupta and Shukla 2021). Newer dopaminergic drugs, immunotherapies, DR, drugs that target neurotransmitters other than dopaminergic ones, regenerative therapies, and deep brain stimulation are among the most recent PD therapeutic approaches. Numerous drugs are currently undergoing clinical trials. Several drugs, such as the following, are being repurposed for PD: Doxycycline is an antibiotic that was formerly thought to be a potential anti-AD treatment strategy. It has since been studied for its anti-PD properties (Meijide et al. 2021). The differentiation between antibacterial and anti-inflammatory actions is based on varying the concentration of doxycycline. Studies have shown that anti-inflammatory action at concentrations lower than those used to treat germs with antibiotics is linked to their neuroprotective benefits but does not affect bacterial susceptibility. Two additional ways that doxycycline aids in neuroprotection are its antioxidant properties and its ability to convert early species of α-synuclein oligomers (a presynaptic neuronal protein linked to PD genetically and neuropathologically) into non-toxic and non-seeding species (Meijide et al. 2021). Research indicates that only oligomeric forms of α-synuclein bind to doxycycline; the physiological monomeric forms of the protein remain unaffected. PD-specific repurposing drugs are shown in Table 2. Research has been conducted on the anti-PD activity of antiasthma medicines, particularly β2-adrenoreceptor agonists.

According to recent studies, the regulation of the SNCA (synuclein alpha) gene has been linked to the β2-adrenoreceptor. More specifically, it has been shown that activation of the β2-adrenoreceptor exhibits neuroprotection. After three anti-asthmatic medications were studied, salbutamol showed the most promise as it had the highest blood-brain barrier permeability. According to Mittal and his group, the analysis undertaken showed that all three drugs could reduce the abundance of α-synuclein and SNCA-mRNA (Mittal et al. 2017).

Huntington's Disease (HD)

Dementia, mental and behavioral problems, and involuntary choreatic movements are characteristics of Huntington's disease (HD) (McColgan and Tabrizi 2018). A genetic mutation results in the development of a mutant form of the multifunctional protein huntingtin (HTT), which is toxic and causes neuronal death and dysfunction. HD arises from CAG (C-cytosine, A-adenine, and G-guanine) trinucleotide DNA segment elongation, repetition, and diversity, which are caused by a mutation in the HTT gene's exon 1 on chromosome 4p16.3. The CAG section is normally repeated 10–35 times in a gene. However, more than 36 CAG repeats are created due to mutations, leading to the origin of HD (Tabrizi et al. 2020).

The onset of HD is indicated by the gradual degeneration of neurons in the basal ganglia, specifically the putamen and caudate nucleus, to the cerebral cortex (Kshirsagar et al. 2021). Adults first experience the symptoms of HD, which get worse over time and eventually cause death within a few years. Since there is no recognized treatment for this ailment, the only option is to manage the symptoms (Roos 2010). Tetrabenazine was first developed as a result of a study into the synthesis of simple

Table 2. Some examples of repurposed drugs for PD ⏎

Drug	Repurposed	Earlier Used	References
Methylphenidate	Numerous trials have shown that this medicine helps reduce non-motor symptoms and gait issues associated with PD.	CNS stimulant used in attention-deficit hyperactivity disorder	(Devos et al. 2013)
Amantadine	It works by increasing dopamine and blocking its reuptake to treat PD. It is a weak glutamate receptor antagonist.	Anti-influenza	(Lee and Kim 2016)
Exenatide	It has demonstrated the ability to cause advantageous neuroplastic changes and neuroprotection, which can halt or slow the disease's course. Through activating GLP-1 receptors, it provides neuroprotection and can pass the blood-brain barrier.	The glucagon-like peptide (GLP) used in type II diabetes	(Jankovic 2017)
Zonisamide	A decrease in intracellular dopamine was observed with increasing dosages. This medicine has shown promising results in treating both motor and non-motor symptoms, while its precise mode of action is unknown.	Antiepileptic	(Fox et al. 2018)
Nilotinib	It was discovered that both elevated α-synuclein expression and build-up are indicators of Abl activation in neurodegeneration. Nilotinib inhibits Abl phosphorylation, which speeds up the breakdown of α-synuclein.	Tyrosine kinase Abl inhibitors, used in chronic myeloid leukemia	(Pagan et al. 2016)

medications having an antipsychotic effect similar to that of reserpine. It works as a highly selective, reversible inhibitor of monoamine absorption by presynaptic neurons as well as a moderate blocker of the D2 dopamine postsynaptic neurons. Due to conflicting research, this substance's antipsychotic potential was abandoned, and now it is repurposed to treat HD, which is characterized by abnormal, involuntary hyperkinetic movements. Compared to dopamine receptor blockers, tetrabenazine is a safer medication to use in HD because it has never been demonstrated to cause dyskinesia (Paleacu 2007).

Numerous drugs with a dopamine antagonistic effect have been studied for the treatment of HD. This is the case with the D2 receptor antagonist antipsychotic medication tiapride. In Europe, selegiline is a commonly used medication to treat Huntington's chorea (Roos et al. 1982). Schizophrenia is treated with clozapine, a neuroleptic drug. It has a strong affinity for the D1 and D4 dopamine receptors but limited antagonistic activity towards the D2 dopaminergic receptors. Due to its low

frequency of extrapyramidal side effects, it was suggested as a good symptomatic therapy for Huntington's chorea despite the mixed results of clinical trials (Bonuccelli et al. 1994). Olanzapine, a different antipsychotic medication, is commonly suggested to address the behavioral and motor symptoms of HD. This drug has a strong affinity for serotonin receptors but is antagonistic to dopamine D2 receptors. Due to its safety and good tolerance, it may be recommended in cases of irritation, insomnia, weight loss, and Huntington's chorea (Paleacu 2007). The antipsychotic risperidone, which is used to treat bipolar disorder and schizophrenia, can also be used to treat HD because it is a serotonin agonist and D2 receptor antagonist. According to Duff and his team, it showed promising outcomes in stabilizing mental symptoms and motor decline. The atypical antipsychotic quetiapine binds to serotonin and dopamine receptors very strongly (Duff et al. 2008). The few cases where quetiapine has been utilized to treat HD symptoms have highlighted the drug's usefulness in treating Huntington's chorea, especially when it is combined with mental symptoms (Alpay and Koroshetz 2006). AD is treated with memantine, a derivative of adamantane. It is a non-competitive N-methyl-Daspartate (NMDA) inhibitor. Excessive activation of the NMDA receptor causes a significant flow of calcium into the cell, which ultimately leads to cell death. Memantine can prevent both the loss of brain cells and this calcium influx in neuronal cells. Memantine has been proven to be useful in treating HD because it can reduce neurons' sensitivity to glutamate-mediated excitotoxicity (Beister et al. 2004). A list of medications used for HD purposes is shown in Table 3.

Table 3. Some examples of repurposed drugs for HD ⏎

Drug	Repurposed	Earlier Used	References
Olanzapine	It is frequently recommended for treating behavioral and motor symptoms of HD. This drug is antagonistic to dopamine D2 receptors despite having a high affinity for serotonin receptors.	Antipsychotic drug	(Paleacu 2007)
Clozapine	Because of its low frequency of extrapyramidal side effects, it was suggested as a good symptomatic drug for HD despite the mixed results of clinical trials.	Neuroleptic drug	(Bonuccelli et al. 1994)
Memantine	Research on the effectiveness of memantine in treating HD showed that it may lessen neurons' susceptibility to glutamate-mediated excitotoxicity.	AD	(Beister et al. 2004)
Risperidone	As a serotonin agonist and D2 receptor antagonist, it is used to treat HD as well as schizophrenia and bipolar disorder.	Antipsychotic drug	(Duff et al. 2008)
Tetrabenazine	It has been repurposed to treat HD symptoms. It works as a high-affinity, reversible inhibitor of monoamine uptake by presynaptic neurons and a moderate blocker of D2 dopamine postsynaptic neurons.	Antipsychotic drug	(Paleacu 2007)

Table 4. Some examples of repurposed drugs for MS and ALS ⏎

Drug	Repurposed	Earlier Used	References
Tamoxifen	Tamoxifen was accidentally repurposed for the treatment of ALS after it was found that people with ALS who had breast cancer treated with the medicine exhibited neurological improvements and disease stability.	An antiestrogen drug used in breast cancer	(Chen et al. 2020)
Amiloride	Amiloride can block the neuroprotective and myeloprotective actions of neuronal proton-gated acid-sensing ion channel 1 (ASIC1), which is overexpressed in oligodendrocytes and axons in MS lesions. Amiloride makes it active even before inflammation starts, which is an additional advantage of its preventive effect that takes place later in the course of inflammation.	Diuretic	(Arun et al. 2013)
Masitinib	Tyrosine kinase inhibitors are used to treat ALS because they may be useful in combating the abnormal glial cells that proliferate in this condition.	Tyrosine kinase inhibitors used in canine cancer	(Trias et al. 2016)
Cyclophosphamide	Because of its potential for immunosuppressive and immunomodulatory effects, cyclophosphamide is utilized in MS treatment. Furthermore, cyclophosphamide can pass the blood-brain barrier and has good absorption in the central nervous system.	An alkylating agent used in breast cancer, acute leukemia, lymphoma	(Awad and Stüve 2009)
Retigabine	It can increase motor neuron survival and decrease excitability, which is advantageous in the treatment of ALS since it is thought that neurons in this condition are hyper-excited and fire more frequently than usual, ultimately leading to cell death.	Anti-epileptic	(Wainger et al. 2021)
Ibudilast	Ibudilast can stop tumor necrosis factor from being released by the brain's astrocytes and microglia, hence reducing neuronal degeneration. Because it can reduce astrocyte death, stop demyelination, and prevent oligodendrocyte apoptosis, it is also beneficial in MS.	Phosphodiesterase inhibitors used in cerebrovascular disorders and bronchial asthma	(Barkhof et al. 2010)

Triumeq® (lamivudine+ dolutegravir + abacavir)	This medication was examined for the treatment of ALS because ALS patients exhibited reverse transcriptase blood concentrations similar to those of HIV patients and because a human endogenous retrovirus was discovered to be expressed in the brains of people living with ALS.	Antiretroviral used in Anti-HIV therapy	(ClinicalTrials.gov 2022)
Mitoxantrone	An anthracenedione used in prostate and breast cancer, lymphoma, and acute leukemia	Because of mitoxantrone's immunosuppressive qualities, which are linked to the central nervous system's T- and B-cells' different responses to antigens, macrophage-caused myelin breakdown, and axonal lesions, the medication has also been approved for the treatment of MS.	(Fox 2004)

Amyotrophic Lateral Sclerosis (ALS) and Multiple Sclerosis (MS)

Amyotrophic lateral sclerosis (ALS) is a disorder that causes the death of both upper and lower motor neurons, which control voluntary muscles. This ultimately results in the weakening and shrinkage of muscles, or muscular atrophy. Additional indicators include trouble speaking, breathing, swallowing, and twitching or tight muscles. Roughly 10% of ALS cases are attributed to genetic inheritance; the majority of ALS causes are unknown (Kiernan et al. 2011). Only two drugs are now available to delay the onset of the disease: riluzole and edaravone. However, once symptoms start, they cannot be reversed (Sawada 2017; Zoccolella et al. 2007).

Multiple sclerosis (MS) is another autoimmune disease that affects the central nervous system. It is a long-lasting inflammatory condition where axons and myelin are completely or partially destroyed. Its early signs include transient neurological abnormalities that ultimately worsen; its development is unknown. Although there is no recognized cure for MS, several drugs can lessen the symptoms and course of the illness (Kakoti et al. 2022). Many medications are presently being repurposed to treat both MS and ALS. A few medicines undergoing clinical trials for ALS or MS are shown in Table 4.

Clinical Perspective

From the perspective of clinical trials, affirmatory outcomes are relatively low on account of failure during Phase II or III trials. However, post-marketing surveillance by many researchers and pharmacological companies often comes with new, unexpected results. Some drugs usually display unanticipated adverse effects, while others are effective against disease conditions that are not originally indicated on their labels. The strategy of DR has emerged as an outcome of these findings. As an example, apomorphine, used initially to treat PD, was repurposed for erectile dysfunction. Similarly, drospirenone was originally designed as an oral contraceptive but was effective against hypertension. Many such drugs are being repurposed to discover new interventions against numerous diseases based on such clinical insights (Pushpakom et al. 2019; Xue et al. 2018).

Challenges and Future Prospective

Drug repositioning has become an essential strategy for using the novel therapeutic potential of existing medications or drug candidates due to its effectiveness in saving time and money. No doubt, using such a clever strategy will speed up the medication development process. Concurrently, while repositioning medications for neurological illnesses, some limitations must be taken into account. The repurposing of drugs faces enormous obstacles, which restricts the market for repurposed treatments. It can cost as much as $300–400 million for a single Phase III clinical study of a medicine that has been repurposed for AD (Shineman et al. 2014). This indicates that while repurposed medications may avoid the original safety and development phase, they still require extensive, high-risk, costly clinical studies to prove their effectiveness. Clinical trials may take a lengthy time due to the slow course of NDs (Kakoti et al.

2022). In addition, medications frequently need to be examined for safety concerns in elderly individuals, who often have comorbidities and receive treatments that could interact with the repurposed drug. This is in addition to demonstrating the drug's penetration into the brain. Because DR involves intricate molecular and cellular signaling pathways, it may be challenging in neuropathological conditions. Drugs that impact one target but respond to many targets may provide an increased risk of various adverse events (Vogt and Mestres 2010). A comprehensive assessment of the benefits combined with the absence of these side effects can help us approach drug repositioning from a wider angle (Reddy and Zhang 2013).

Additional difficulties in repurposing medications include commercialization, reimbursement issues, and little to no patent protection. We can take into consideration a number of suggestions to get over obstacles that arise throughout the DR process. First and foremost, it is necessary to provide more funding for clinical trials of medications intended for repurposing. Unless there is a clear commercial strategy, pharmaceutical corporations are unlikely to fund human trials for authorized treatments that need to be repositioned. This requires the governments and foundations to take the initiative. At the moment, several organizations are quite interested in sponsoring Phase I studies of repurposed medication candidates in an effort to encourage DR (Kakoti et al. 2022).

Repurposing trials have been funded by several organizations, including the Multiple Myeloma Research Foundation, the Alzheimer's Society (United Kingdom), the Cure Parkinson's Trust, the MJFF, and others (Kakoti et al. 2022). Various academic centers are also leading the repurposing efforts along with government initiatives. Secondly, establishing a comprehensive data analysis platform is essential to enhance data sharing. The vast data generated by authorized medications or drug candidates undergoing clinical trials can be organized and re-examined through artificial intelligence and information science. The research process is slowed down by the immense and complex amounts of data from human trials and biological databases that are beyond the capabilities of traditional data processing techniques. Without a doubt, this enormous data will help us to understand the condition better and develop more precise disease-related tactics. However, there's a big gap between data analysis and biomedical data production of DR. Experts must find technical clarifications to guarantee research efficiency while consuming less time and energy. Ultimately, it is essential to overcome patent limits and execute judicious surveillance to facilitate the DR process. The application of DR needs to be supported by a risk management plan, and clinical trial data or post-marketing surveillance data can verify the medication's safety confirmation (Kakoti et al. 2022).

Conclusion

In conclusion, when integrated with AI and other advanced computational techniques, DR can potentially revolutionize the treatment of NDs and other complex conditions. By accelerating the identification of new therapeutic uses for existing drugs, this approach can lead to more effective and timely treatments, ultimately improving therapy outcomes. As was previously said, many businesses are looking into the strategic benefits of employing AI and ML-based frameworks for drug discovery in

this market. However, to fully realize the potential of DR, it is essential to address the challenges associated with AI interpretability, enhance the accuracy of computational methods, and ensure robust experimental validation. The accurate evaluation of available preclinical and clinical data is still a challenge despite these developments, especially when looking at it from a scientific and regulatory standpoint. In addition to concentrating on the efficacy of newly repurposed medications, thorough post-authorization studies remain crucial. As these challenges are overcome, DR is poised to play an increasingly important role in the future of medicine, offering new hope for patients with unmet medical needs.

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CHAPTER

3

Drug Repurposing for Psychiatric Diseases

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Introduction

Psychiatric diseases are syndromes characterized by disturbances in cognition, emotion regulation, and behavior (American Psychiatric Association 2013). They involve various disorders, including depression, schizophrenia, attention deficit hyperactivity disorder (ADHD), autism spectrum disorder (ASD), anxiety disorder, bipolar disorder, epilepsy, post-traumatic stress disorder, eating disorder, disruptive behavior and dissocial disorder, and substance use disorder (Collins et al. 2011; WHO 2022). Psychiatric diseases have a significant impact on public health, with one in eight people suffering from some form of psychiatric disease worldwide (WHO 2022). Depression, anxiety disorder, schizophrenia, ASD, alcohol use disorder (AUD), and drug use disorder accounted for 6.2, 4.7, 1.7, 1.3, 1.2, and 1.0% of all-cause years lived with disability (YLDs), which refers to the total number of years that people live with a disability in 2021, representing the 2nd, 6th, 18th, 21st, 22nd, and 24th highest causes of global YLDs, respectively (GBD 2021; Diseases and Injuries Collaborators 2024). Despite the huge burden of these diseases, treatment options and their efficacies remain limited. Available drug mechanisms for the treatment of depression are limited, including the increase of serotonin (5-hydroxytryptamine, 5-HT), norepinephrine, and/or dopamine by tricyclic antidepressants, monoamine oxidase inhibitors, selective serotonin reuptake inhibitors (SSRIs), serotonin and norepinephrine reuptake inhibitors (SNRIs), and noradrenaline and dopamine reuptake inhibitors (Yao et al. 2022). Only 30% of patients respond to the first line of treatment, and approximately 30% treated with two or more first-line antidepressants fail to achieve remission, which is considered treatment-resistant depression (TRD)

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(Kverno and Mangano 2021). For schizophrenia treatment, first-, second-, and third-generation antipsychotic drugs for dopamine D2 receptor blockade, D2 and serotonergic 5-HT2 receptor blockade, and partial agonism of D2 receptor with modification of several 5-HT receptor subtypes have been developed and used in clinical settings (Rybakowski 2023). However, the currently available antipsychotic medications are relatively ineffective against the negative and cognitive symptoms of schizophrenia, representing a significant unmet medical need, and novel action mechanisms have been proposed to overcome these limitations (Pei et al. 2021). For anxiety disorders, SSRIs, SNRIs, and cognitive behavioral therapy are used as first-line therapies (Bandelow et al. 2023), but only 50–67% of patients show adequate clinical responses to these treatments (Domschke et al. 2024). Antipsychotic drugs and SSRIs are used to treat irritability, aggression, and anxiety symptoms in ASD; however, their side effects pose clinical challenges (Nanjappa et al. 2022).

In psychiatric diseases, only a limited number of drug action mechanisms—referring to the specific biochemical molecule or process through which a drug produces its effects in the body—are effective. Furthermore, the same action mechanism is often tested for multiple diseases for various reasons. For example, extrapolation from non-clinical animal models to clinical settings for humans is particularly difficult in psychiatric diseases. This is because animal models do not accurately reflect the human disease status in psychiatric diseases due to the subjective nature of the core symptoms and the influence of environmental factors on disease development (Bravo et al. 2009). However, novel animal modeling approaches have been developed for major depressive disorder (MDD) (Demin et al. 2019). Due to the complex disease pathogenesis and significant differences in the brain structure and function between humans and animals, the efficiency of animal models used for translation from biomedical research to clinical applications is lower in psychiatric diseases than in other diseases (Wendler and Wehling 2017). Moreover, validation of the efficacy and safety of new drug candidates is particularly difficult for psychiatric diseases. The pathogenesis of these diseases is complex and involves multiple mechanisms, including neurotransmitter deficits, inflammation, genetic factors, epigenetics, and the microbiome (Fakhoury 2015; Wang et al. 2022; Zhang et al. 2022; Reus et al. 2023). Patients with psychiatric diseases exhibit heterogeneity, resulting in limited confidence in short-term clinical output to long-term efficacy, thus leading to expensive trials and failures in clinical development (Finn and Constable 2016; Krainc et al. 2023). The placebo effect also hampers the validation of drug efficacy for psychiatric diseases. Placebos cause clinically significant neurobiological changes (Murray and Stoessl 2013) and threaten the success of new drug development for psychiatric diseases (Correll et al. 2023). The potential risk of adverse effects is also a challenge for the development of psychiatric disease drugs. Psychiatric diseases are often chronic, causing adverse effects during their long-term use (Ambwani et al. 2021). The deviation in neurotransmitter levels leads to unwanted behavioral changes (Rajkumar and Melvin 2014).

Drug repurposing approaches are widely used to overcome the difficulties associated with drug development for psychiatric diseases. One-third of the new drugs approved by the U.S. Food and Drug Administration (FDA) for psychiatric diseases over the past twenty years have been developed using drug repurposing approaches

(Okuyama 2023). Drug repurposing reduces the risk of clinical development failure by relying on existing compounds and their information. This technique is especially beneficial for fields such as psychiatric disease treatment, where extrapolation from animal findings to humans is difficult, and variation in human responses to drug treatment is huge due to high disease heterogeneity and placebo effects. Only 9% of drug candidates discovered during the research phase achieve regulatory approval via clinical development in neurology, which is lower than the average success rate in all therapeutic areas (Hay et al. 2014). This chapter focuses on the repurposing of drugs for the treatment of psychiatric diseases. The following section discusses the efficiency of drug repurposing strategies and various repurposing concepts for psychiatric medications. The third section summarizes the approved psychiatric drugs developed using drug repurposing approaches. The fourth section lists the recent drugs for psychiatric diseases developed via drug repurposing currently in the late clinical development stage. The last section highlights the conclusions.

Efficiency of Drug Repurposing in Psychiatric Drug Development

Any new chemical entity discovered in biomedical research poses the risk of its expected efficacy not being translated to clinical settings. Drug discovery researchers often estimate the efficacy of new drug candidates in animal disease models. However, animal models do not accurately reflect the human conditions. Therefore, the risk is exceptionally high for diseases, such as psychiatric diseases, that lack effective animal models translatable to humans (Wendler and Wehling 2017). Disease heterogeneity (Krainc et al. 2023) and large placebo effects (Murray and Stoessl 2013) in patients further increase the difficulty of determining the efficacy of new psychiatric drug candidates in clinical trials. For psychiatric diseases, drug discovery and development through phenotypic approaches, which rely on observations in clinical settings, are more effective than hypothesis-based approaches that depend on the biological and pathophysiological knowledge derived from biomedical research. Therefore, drug repurposing initiatives using existing drugs and their human efficacy information from clinical use are effective for psychiatric drug development.

Additionally, drug repurposing is beneficial for psychiatric diseases with unclear and complex disease mechanisms, which compromise the identification of novel drug targets (Agid et al. 2007). Since the serendipitous discovery of monoaminergic neurotransmission regulation in the 1950s and 1960s, no drugs with fundamentally new action mechanisms have emerged in decades for psychiatric diseases (Lago et al. 2021). Therefore, applying the proven mechanisms and compounds in one psychiatric disease to another increases the likelihood of drug development success over testing new mechanisms and compounds, as the pathophysiological mechanisms of some psychiatric diseases overlap with each other (Caban et al. 2017).

Drug Repurposing Concepts for Psychiatric Drugs

Drug repurposing involves three strategies: repositioning, reformulation, and combination (Caban 2017). Repositioning seeks different applications for drugs

approved or developed for different indications. Reformulation aims to improve the drug profile by changing the route of administration, creating prodrugs and extended-release formulations, and using active metabolites and enantiomers of original drugs. Combination seeks to improve the drug profile by combining two or more existing drugs. Various concepts have been developed for psychiatric drugs by applying these strategies (Okuyama 2023). In some cases, more than one repurposing strategy is used for drug development.

The first concept for repurposing psychiatric drugs involves the determination of new use of existing compounds. Among the approved psychiatric drugs developed via drug repurposing approaches, lofexidine for opioid withdrawal symptoms, esketamine for TRD, viloxazine for ADHD, dextromethorphan in combination with bupropion for MDD, dexmedetomidine for agitation in schizophrenia or bipolar disorders, and cariprazine for MDD are the examples of approved drugs applied to different indications (Urits et al. 2020; Lamb 2021; Tabuteau et al. 2022; Citrome et al. 2022; Abbvie 2022; Boudieu et al. 2023). The development of stiripentol for the treatment of seizures associated with Dravet syndrome is a case in which a new indication was sought for a compound that failed in the late clinical development stage for a different indication (Frampton 2019). Cannabidiol is a natural product used for the treatment of severe epilepsy (von Wrede 2021).

The second concept involves the reduction of the parent drug's side effects. Two drugs listed in the next section fall into this category: paliperidone for schizophrenia, where a sustained-release formulation of the active metabolite of the parent drug (risperidone) was developed to reduce the side effects of the parent drug (Corena-McLeod 2015), and olanzapine and samidorphan, where a clinically-developed compound (samidorphan) was combined with the parent drug used for schizophrenia and bipolar I disorder (olanzapine) to reduce the side effects of the parent drug (Gupta and Singh 2024).

The third concept seeks to improve medication compliance. Four drugs listed in the next section fall into this category: desvenlafaxine and aripiprazole lauroxil, where the dosing frequency was reduced by the prodrug formulation (Seo et al. 2010; Frampton 2017), and serdexmethylphenidate and dexmethylphenidate combination and TV-46000, where symptom control was improved by formulation changes (Braeckman et al. 2022; Kane et al. 2023). Desvenlafaxine, a prodrug of venlafaxine, attenuates drug interactions better than its active form (Seo et al. 2010).

The fourth concept seeks to increase drug efficacy. In the combination of dextromethorphan and bupropion for MDD, the efficacy of dextromethorphan was enhanced by inhibiting its cytochrome P450 (CYP)-2D6-dependent metabolism and adding another antidepressant via combination with bupropion, an approved antidepressant with CYP2D6 inhibitory activity (Tabuteau et al. 2022).

Approved Psychiatric Drugs Developed via Drug Repurposing Approaches

This section highlights the psychiatric drugs developed via drug repurposing and approved by the FDA in the past 20 years.

Paliperidone

Risperidone is an antipsychotic drug used to treat schizophrenia (Möller 2005). Paliperidone is the active metabolite of risperidone. Owing to the slow release of active ingredients by extended-release formulations and differences in 5-HT2A and D2 receptor binding affinities, pariperidone shows a lower prevalence of adverse events, such as extrapyramidal symptoms, compared to risperidone (Corena-McLeod 2015; Takeuchi et al. 2013).

Desvenlafaxine

Venlafaxine is an SNRI used as an antidepressant. However, this drug has several limitations. Its half-life is approximately only 5 h (Shelton 2019). Moreover, venlafaxine is metabolized to its active metabolite by CYP2D6, leading to significant inter-individual variations in CYP2D6 metabolism and drug-drug interactions with other CYP2D6 inhibitors (Shelton 2019). Desvenlafaxine is an active metabolite of venlafaxine that exerts minimal impact on CYP2D6 metabolism (Seo et al. 2010). It is more beneficial than venlafaxine owing to its once-daily dosing with a longer half-life (12 hrs) and steady-state plasma concentrations achieved within 4–5 d, leading to convenient dosing (Seo et al. 2010). Desvenlafaxine has been approved by the FDA for MDD treatment (Seo et al. 2010).

Aripiprazole lauroxil

Aripiprazole lauroxil is an injectable extended-release prodrug of aripiprazole, a clinically established atypical antipsychotic (Frampton 2017). This prodrug was designed to decrease the risk of metabolic side effects of aripiprazole by improving its pharmacokinetics and enabling multiple dosing options (Frampton 2017).

Lofexidine

Lofexidine, an alpha-2 adrenergic receptor agonist, is widely used to treat high blood pressure. It was further repurposed and approved by the FDA in May 2018 for opioid withdrawal symptoms (Urits et al. 2020). Based on the off-label use of clonidine, another alpha-2 adrenergic receptor agonist for opioid withdrawal, lofexidine was clinically tested and proven to be more effective for opioid-dependent patients (Urits et al. 2020; Yu et al. 2008).

Cannabidiol

Cannabidiol is an inverse agonist or antagonist of the cannabinoid-1 receptor and antagonist of the cannabinoid-2 receptor, with partial activity on vanilloid channels and 5-HT1A receptors (Peng et al. 2022). Moreover, cannabidiol is a commonly sold cannabinoid supplement. As cannabidiol improves severe seizures in humans (von Wrede 2021), it was further developed and approved for the treatment of epilepsy in 2018 (Huntsman et al. 2020).

Stiripentol

Stiripentol has several possible molecular targets, including γ-aminobutyric acid

(GABA)-A receptor and lactate dehydrogenase (Nickels and Wirrell 2017). It was originally developed for adults with focal seizures but failed in the phase three trials (Nabbout et al. 2017). Then, it was repurposed and approved for treating seizures associated with Dravet syndrome in Europe, Canada, Japan, and the USA (Frampton 2019).

Esketamine

Ketamine, a dissociative anesthetic, is an N-methyl D-aspartate (NMDA) receptor antagonist, and esketamine, an enantiomer of ketamine, has four times more potent activity against NMDA receptors than the other enantiomers (Zanos et al. 2018). Intravenously administered ketamine leads to a robust and rapid improvement in depressive symptoms in patients with MDD with TRD (Murrough et al. 2013). An intranasal formulation of esketamine has been developed to reduce the side effects of intravenous administration by bypassing the blood-brain barrier (Boudieu et al. 2023). This formulation has been approved by the FDA for patients with MDD with TRD (Traynor 2019).

Serdexmethylphenidate and Dexmethylphenidate

Serdexmethylphenidate and dexmethylphenidate combination combines dexmethylphenidate, an active moiety, with serdexmethylphenidate, a prodrug form of dexmethylphenidate (Braeckman et al. 2022). Methylphenidate is used to treat ADHD, and dexmethylphenidate, an isomer of methylphenidate, is the main pharmacological contributor to its efficacy in ADHD treatment (Quinn 2008). Combination with serdexmethylphenidate improves the pharmacokinetics of dexmethylphenidate, leading to rapid onset and sustained efficacy and contributing to better symptom control (Braeckman et al. 2022).

Viloxazine

An immediate-release formulation of viloxazine, a norepinephrine reuptake inhibitor, has been marketed as an antidepressant in Europe since the 1970s (Chungping et al. 2020). The drug was later withdrawn from the market for business reasons (Faison et al. 2021). Due to the involvement of norepinephrine transmission in the pathophysiology of ADHD (Pozzi et al. 2020), an extended-release formulation of viloxazine was later developed and approved by the FDA for the treatment of pediatric ADHD (Lamb 2021).

Olanzapine and Samidorphan

Olanzapine is a second-generation antipsychotic commonly used to treat schizophrenia and bipolar disorder. However, it exerts side effects, such as weight gain and metabolic abnormalities (Rehan et al. 2022). Samidorphan, a clinically developed and subsequently withdrawn compound, is an antagonist of the mu-opioid receptor and a partial agonist of the kappa and delta opioid receptors (Chaudhary et al. 2019). The antagonism of the mu-opioid receptor inhibits the reward system; therefore, samidorphan is used in combination with olanzapine to prevent its

metabolic side effects (Gupta and Singh 2024). This combination of drugs reduces weight gain and metabolic abnormalities, facilitating the effective management of schizophrenia and bipolar disorder (Gupta and Singh 2024).

Dextromethorphan and Bupropion

Dextromethorphan is widely used as an antitussive agent; however, it is quickly metabolized by CYP2D6 (Nguyen et al. 2016; Silva and Dinis-Oliveira 2020). It was expected to act as an antidepressant owing to its NMDA antagonism activity; however, a short half-life limits its use (Schatzberg 2022). To address this issue, bupropion, a clinically used antidepressant and a CYP2D6 inhibitor (Foley et al. 2006; Kotlyar et al. 2005), has been combined with dextromethorphan to prolong its half-life and exert potent antidepressant effects (Tabuteau et al. 2022).

Dexmedetomidine

Dexmedetomidine is an alpha2-adrenergic agonist that exerts minimal effects on the hemodynamic profile and is clinically used for sedation analgesia (Wang et al. 2024). This drug has been used for the acute treatment of agitation associated with schizophrenia or bipolar disorders based on the discovery by EvolverAI, an artificial intelligence platform for the reuse of approved drugs in a new form or indication (Bioxcel Therapeutics 2021, 2024). Its reformulation as a sublingual film also facilitates its use to manage agitation in psychiatric settings (Citrome et al. 2022). This new formulation of dexmedetomidine was approved by the FDA in 2022 (Citrome et al. 2022).

Cariprazine

Cariprazine is a D3/D2 dopamine receptor partial agonist with a higher affinity for D3 (Keks et al. 2020). It has been approved for the treatment of schizophrenia and manic, mixed, and depressive episodes associated with bipolar I disorder (Do et al. 2021). Cariprazine was later repositioned for MDD and showed significant changes in the Montgomery-Åsberg Depression Rating Scale (MADRS) total score. In 2022, it was approved by the FDA as an adjunctive treatment for MDD (Abbvie 2022).

TV-46000 (Extended-Release Risperidone)

Risperidone is an atypical antipsychotic widely used to treat schizophrenia and bipolar disorder. TV-46000 is a long-acting subcutaneous form of risperidone that uses a copolymer-based drug delivery technology (Kane et al. 2023). This compound showed a significant delay of impending relapse of schizophrenia in a phase three study, highlighting its potential as an effective treatment option with a favorable benefit-risk profile for schizophrenia (Kane et al. 2023). TV-46000 was approved by the FDA in 2023 (Teva 2023).

Compound names, repurposing applications and strategies, and development concepts of 14 drugs discussed in this section are summarized in Table 1.

Table 1. A list of US Food and Drug Administration (FDA)-approved psychiatric drugs obtained through drug repurposing approaches ⏎

Compound Name	Application	Repurposing Strategy	Development Concept
Paliperidone	Schizophrenia	Reformulation	Reduction of the parent drug side effects
Desvenlafaxine	MDD	Reformulation	Improvement in medication compliance of the parent drug
Aripiprazole lauroxil	Schizophrenia	Reformulation	Improvement in medication compliance with the parent drug
Lofexidine	Opioid withdrawal symptoms	Repositioning	New indication for approved drug
Cannabidiol	Epilepsy	Repositioning	New indication for a natural compound
Stiripentol	Seizures associated with Dravet syndrome	Repositioning	New indication for clinically developed but failed compound
Esketamine	TRD	Repositioning Reformulation	New indication for approved drug
Serdexmethylphenidate and dexmethylphenidate	ADHD	Reformulation Combination	Improvement in medication compliance with the parent drug
Viloxazine	ADHD	Repositioning Reformulation	New indication for approved drug
Olanzapine and samidorphan	Schizophrenia Bipolar I disorder	Combination	Reduction of the parent drug side effects
Dextromethorphan and bupropion	MDD	Repositioning Combination	New indication for approved drug and increase in the parent drug efficacy
Dexmedetomidine	Agitation associated with schizophrenia and bipolar disorders	Repositioning Reformulation	New indication for approved drug
Cariprazine	MDD	Repositioning	New indication for approved drug
TV-46000 (extended-release risperidone)	Schizophrenia	Reformulation	Improvement in medication compliance with the parent drug

Drug Repurposing Approaches in Late Development Stage

Drug repurposing approaches for various psychiatric diseases are currently in the late clinical development stage. Here, some examples of drugs currently undergoing and recently withdrawn from phase three trials are provided for diseases for which drug repurposing is being actively pursued.

MDD

Methadone is a synthetic opioid agonist used for analgesia in chronic pain and opioid use disorders (Anderson and Kearney 2000). Esmethadone is an enantiomer of methadone exhibiting NMDA antagonism (Codd et al. 1995). Esmethadone is currently in phase three development for MDD treatment (Fava et al. 2023).

Psilocybin, a natural tryptamine compound in some mushroom species, is used for healing and spiritual purposes (Hristova and Perez-Jover 2023). It exerts serotonergic and glutamatergic effects (Vollenweider and Kometer 2010). The preliminary evidence of antidepressant effects of psilocybin has been demonstrated in psilocybin-assisted therapy (Griffiths et al. 2016). The efficacy of psilocybin for MDD treatment has been confirmed in randomized clinical trials (Hristova and Perez-Jover 2023), and this compound is currently undergoing phase three trials (Compass Pathways 2023).

Despite the progress in this field, some drug repurposing approaches for MDD have failed in recent years. Pimavanserin is an atypical antipsychotic with an inverse agonism and antagonism of 5-HT2A and 2C receptors (Vanover et al. 2006). It has been approved for psychosis in Parkinson's disease. However, when clinically tested for MDD, this drug did not exhibit any positive effects, leading to the discontinuation of its repurposing for MDD in 2020 (Scala et al. 2023). Buprenorphine is a kappa opioid agonist clinically used for pain and opioid-use disorders (Spinella and McCarthy 2024; Wakhlu 2009). Samidorphan is a mu-opioid antagonist used in combination with olanzapine to treat schizophrenia and bipolar depression (Chaudhary et al. 2019). The buprenorphine and samidorphan combination was tested in phase three trials for MDD as an add-on therapy (Machado-Vieira et al. 2017); however, its clinical efficacy was insufficient, leading to its rejection for MDD treatment in 2019 (Reuters 2019).

Schizophrenia

KarXT is a combination of xanomeline (muscarinic M1 and M4 receptor agonist) and trospium (peripheral muscarinic receptor antagonist). Xanomeline showed promising efficacy for schizophrenia in clinical studies but failed at the clinical development stage due to gastrointestinal side effects caused by its peripheral muscarinic agonism (Shekhar et al. 2008). Trospium is an approved peripheral muscarinic antagonist that does not penetrate the blood-brain barrier (Rovner 2004). Hence, the combination of xanomeline and trospium in KarXT shows good efficacy with reduced intestinal side effects in patients with schizophrenia (Karuna Therapeutics Inc. 2024). KarXT

has shown promising results in phase three trials (Brannan et al. 2021), with new applications being assessed by the FDA (Karuna Therapeutics Inc. 2023).

Cannabidiol, initially used as a supplement, was repositioned for epilepsy (see the fifth paragraph of the third section). It exerts multiple pharmacological effects. It has shown promising efficacy against schizophrenia cognitive deficits in preclinical models; however, its effects on the cognitive functions of patients with schizophrenia were not potent in small-scale clinical studies (Norman 2024). Currently, cannabidiol is being tested for early schizophrenia in phase three trials (ClinicalTrials.gov 2023).

Estrogens are possibly the next line of treatment for schizophrenia. Estradiol, a sex steroid used in hormone therapy, improves cognitive function in women with schizophrenia (Ko et al. 2006). Recent clinical trials on estradiol have demonstrated the improvement of positive symptoms on the Positive and Negative Syndrome Scale (PANSS) in patients with schizophrenia (Weiser et al. 2019), and many ongoing trials are in phase three (Correll 2023). Raloxifene, a selective estrogen receptor modulator used for osteoporosis, is effective for schizophrenia treatment (Mu et al. 2024). One phase three trial showed PANSS improvement (Usall et al. 2016), whereas another did not support the use of raloxifene for schizophrenia (Brand et al. 2023). The research on this drug is ongoing (Mu et al. 2024).

As mentioned in the first paragraph of the fourth section, pimavanserin is an atypical antipsychotic clinically used for the treatment of psychosis in Parkinson's disease. It was also proposed for schizophrenia treatment. However, it did not significantly improve the negative symptoms of schizophrenia in a phase three trial, leading to the discontinuation of its tests for schizophrenia treatment (ACADIA 2024).

Bipolar Depression

Interestingly, promising compounds for bipolar depression in phase two and three trials have all been repurposed candidates (Correll et al. 2023). Specifically, two compounds are in phase three trials. Armodafinil, an enantiomer of modafinil with a long half-life, was approved in 2007 for treating excessive sleepiness (Lankford 2008). This drug improves treatment-resistant bipolar depression (Kwok et al. 2023). Another drug currently in phase three trials is ketamine. Ketamine, an NMDA receptor antagonist and a dissociative anesthetic, shows efficacy against TRD, and its S-enantiomer (esketamine) has been approved for treating TRD (Jawad et al. 2022). The efficacy of ketamine for bipolar depression treatment has been tested in clinical settings (Jawad et al. 2023).

Substance Use Disorders

Until 2023, three compounds were in Phase III trials for substance use disorder, including one for AUD and two for amphetamine and methamphetamine use disorder (Correll et al. 2023). All three compounds are repurposed drugs.

Baclofen is a selective GABA-B receptor agonist used for muscle relaxation. Baclofen is often used off-label in clinical practice to treat AUD. Although its use for AUD has been investigated in randomized control trials, its clinical evidence remains inconsistent (Beaurepaire et al. 2019).

To date, no pharmacotherapy has been approved for the treatment of amphetamine and methamphetamine use disorder. Mirtazapine, an atypical antidepressant with mixed monoamine agonist/antagonist activity, has been tested for amphetamine and methamphetamine use disorder (Naji et al. 2022). This drug increases norepinephrine, serotonin, and dopamine levels in the brain region, mediating the rewarding responses; it drives cravings and drug-seeking behavior (Coffin et al. 2020). An extended-release, fixed-dose combination drug containing naltrexone, an opioid antagonist, and bupropion, an aminoketone antidepressant, is used to treat obesity (Sherman et al. 2016). The efficacy of the naltrexone and bupropion combination for methamphetamine use disorder treatment has been tested in clinical trials (Trivedi et al. 2021).

Compound names, repurposing applications and strategies, and development concepts for the above-mentioned drugs that are currently undergoing clinical trials are summarized in Table 2.

Table 2. Examples of drugs for psychiatric diseases obtained through repurposing that are currently in late-stage development ⏎

Compound Name	Application	Drug Repurposing Strategy	Development Concept
Esmethadone	MDD	Repositioning Reformulation	New indication for approved drug
Psilocybin		Repositioning	New indication for a natural compound
KarXT	Schizophrenia	Repositioning Combination	New indication for clinically developed but failed compound and reduction in the parent compound side effects
Cannabidiol		Repositioning	New indication for natural compound and approved drug
Estradiol		Repositioning	New indication for approved drug
Raloxifene		Repositioning	New indication for approved drug
Armodafinil	Bipolar depression	Repositioning Reformulation	New indication for approved drug
Ketamine		Repositioning	New indication for approved drug
Baclofen	AUD	Repositioning	New indication for approved drug
Mirtazapine	Amphetamine and methamphetamine use disorder	Repositioning	New indication for approved drug
Naltrexone and bupropion		Repositioning	New indication for approved drug

Conclusion

Repurposing of existing drugs is actively used for new drug development for psychiatric diseases. The drug repurposing approach is widely used owing to the difficulty in reflecting the human pathological conditions in animal models and the complexity and ambiguity of psychiatric disease mechanisms, which make the identification of new targets challenging. In psychiatric drug development, drug repurposing strategies involving repositioning that explores the application of existing compounds to different diseases, reformulation that improves the drug profiles by changing the formulation of existing drugs, and combination that enhances the drug profiles by combining multiple compounds are widely used for various purposes, including new application development, enhanced drug safety and efficacy, and increased medication compliance. Over the past 20 years, approximately 14 drugs approved by the FDA for psychiatric disease treatment were obtained via drug repurposing. Many repurposing approaches, both promising and failed, have mainly targeted depression, schizophrenia, bipolar disorder, and substance use disorder. In conclusion, this chapter highlights the importance of drug repurposing for psychiatric disease treatment and encourages repurposing efforts in the future.

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CHAPTER
4

Drug Repurposing as a Novel Approach to Cancer Treatment

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Introduction

Drug repurposing, sometimes referred to as drug repositioning, is the process of finding new pharmacological applications for a medication that has already received a license and is being sold for treating other diseases (Sleire et al. 2017; Pushpakom 2022; Sperry and Donald 2024). This approach is especially advantageous when therapy is urgently required because it leverages the pharmacokinetic and toxicological data from pre-clinical and post-clinical trials of these drugs. It is especially helpful when conventional *de novo* drug design and development approaches are not financially feasible.

Many pharmaceutical firms are focusing on implementing research on drug repurposing to treat different types of cancers. Given the global cancer burden and high treatment costs, repurposed drugs offer a cost-effective alternative, potentially improving access to cancer care and reducing the need for new drug development. The traditional process of drug discovery and development can take anywhere from 10 to 17 years, encompassing research, preclinical testing, clinical trials, and regulatory approval. In contrast, drug repurposing can significantly shorten this timeline by entering the process at the Phase I clinical trial stage, potentially saving upto 7 years that would typically be spent on early research, as depicted in Figure 1.

Certain drugs developed for targeted or organ-specific cancers are also being repurposed to treat other tumor types because many cancers share common molecular pathways, receptors, or signaling mechanisms. For example, imatinib was developed for chronic myelogenous leukemia and has been repurposed for gastrointestinal stromal tumors. Everolimus, initially approved for renal cancer, is now used for pancreatic cancer, while docetaxel and paclitaxel, originally approved for prostate and ovarian cancer, have been repurposed for breast cancer (Aggarwal et al. 2021).

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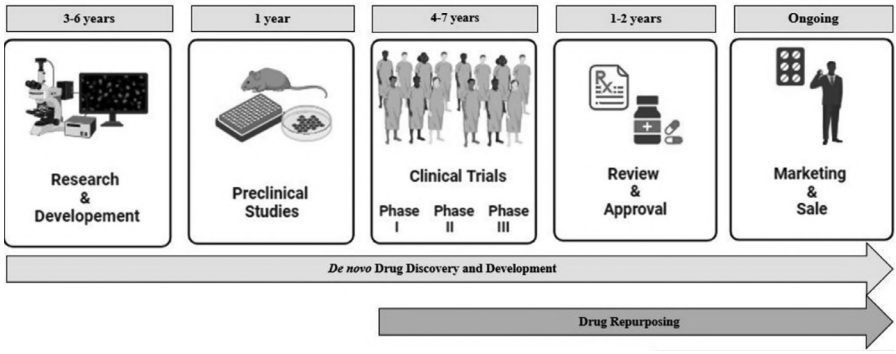

Figure 1. Comparative lifespan for drug discovery and drug repurposing. ⏎

This book chapter provides an overview of novel potential targets and relevant signaling pathways of repurposed drugs in cancer therapies, along with a discussion of various designing approaches that contribute to drug repurposing. These promising findings offer valuable insights into repurposing non-cancer drugs for cancer drug discovery.

Emerging Trends and Innovative Approaches in Repurposing Drugs and Their Development

Repurposed drugs are developed through drug-centric, disease-centric, and target-centric approaches, as illustrated in Figure 2. Each approach explores the relationship between drug, disease, and targets according to the therapeutic effect of a drug (Parisi et al. 2020).

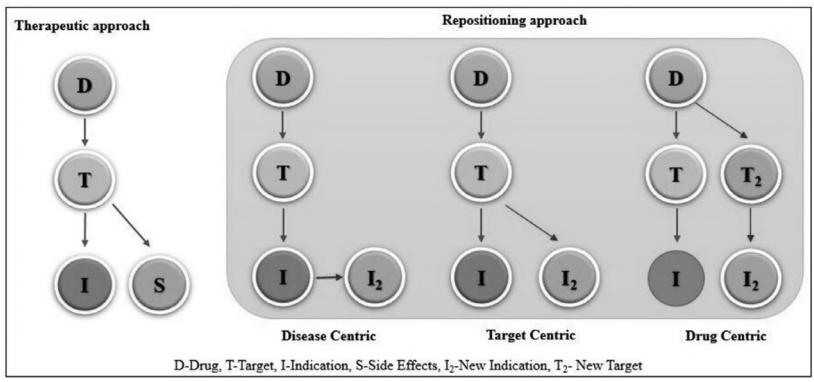

Figure 2. Three approaches for drug repurposing. ⏎

Drug Centric

A drug-centric approach emphasizes finding new indications for an approved drug that targets different patient groups or medical conditions beyond its current licensing

or patent. It can also repurpose drugs that were removed from the market due to safety or post-market issues, provided they retain efficacy for alternative applications. Additionally, this strategy can explore new applications for drugs whose patents have expired and now face competition from generic versions. For example, valproic acid is traditionally used for seizures and bipolar disorder as it binds to the enzymes in the mitochondria; however, it does have an off-target interaction with a protein involved in several cancer types (Parisi et al. 2020). Valproic acid has been repurposed for cancer therapy because it may arrest cancer cell growth and cause apoptosis. Moreover, testing drugs for specific diseases without detailed biological information often leads to serendipitous discoveries and is referred to as the phenotypic drug screening approach (Jin and Wong 2014; March-Villa 2017). Though it does not clarify mechanisms of action, it allows broader application across diseases, as seen with etoposide, repurposed for bladder cancer (Munk et al. 2007).

Disease Centric

A disease-centric approach links approved or unsuccessful drugs with rare diseases that have no treatment or a medication which is only partially effective. For instance, nilotinib, a kinase inhibitor, is now being repurposed for treating gastrointestinal stromal tumors (GIST) instead of Philadelphia chromosome-positive chronic myelogenous leukemia (Blay et al. 2015; Malla et al. 2024). It functions by preventing the platelet-derived growth factor receptor alpha (PDGFRα) gene or proto-oncogene of stem cell factor receptor (KIT) from activating its tyrosine kinase activity and thus interrupting the oncogenic signaling pathway and tumor growth.

Additionally, *in-silico* screening approaches, including artificial intelligence and neural or pathway network approaches, have been utilized to reconstruct the disease-specific routes from proteomic, genomic, and genetic data. For instance, Zhang et al. (2014) built a disease-similarity network using gene expression profiles to suggest drug repositioning opportunities with an example of the commonly used antidiabetic drug metformin, evaluated as a therapy for Alzheimer's disease.

Target Centric

This approach focuses on identifying specific molecular targets involved in the pathophysiology of diseases and employing current drugs that affect these new targets. For instance, the NSAID celecoxib, initially accepted for treating adult rheumatoid arthritis and osteoarthritis, has been repurposed for cancer treatment. As a COX-2 target inhibitor, celecoxib has shown the potential to lower the possibility of metastasis among patients with cancer, especially in a preoperative setting (Mohsin et al. 2022). Another example is chlorpromazine, a typical antipsychotic (Anagnostis et al. 2024) that interacts with the human serotonin 2A receptor (HTR2A), affecting both antiemetic and tranquilizer actions (Parisi et al. 2020). The target-centric approach utilizes bioinformatics and cheminformatics tools to assess the chemical structures, drug-target networks, drug-protein interactions, and data from clinical trials via molecular docking, gene signature, and machine learning processes. For example, remdesivir has been identified through a molecular docking approach as a potential inhibitor of the Nsp12 active site (non-structural protein in coronavirus

gene) received FDA emergency approval for treating COVID-19 infection (Fadlalla et al. 2022). Another drug, sildenafil, was originally developed for hypertension; now, it is repurposed for erectile dysfunction due to its significant benefits through a gene signature analysis approach (Ghofrani et al. 2006). Similarly, using the same gene signature analysis approach, sirolimus was repurposed for dexamethasone-resistant acute lymphoblastic leukemia (Lamb et al. 2006).

New Window of Opportunity for Cancer Therapy Using Drug Repurposing Approach

Cancer is a globally prevalent and deadly disease, with projections of 29.4 million new patients by 2024 and 9.9 million fatalities in 2020 (Bray et al. 2024). Cancer cells produce a distinct microenvironment of tumors in which cancer stem cells (CSCs) can proliferate new tumors. CSCs demonstrate the ability to be multi-lineage and self-renewing. After CSCs split, some of their progeny differentiate, while other CSCs undergo further mutations that lead to the development of more invasive forms of CSCs that eventually migrate to neighboring tissue, and metastasis takes place. Therefore, as seen in solid tumors, such as cancer fibroblasts, additional stromal cells are mostly produced from CSCs. However, some normal cells also migrate into the cancerous area; for example, fibroblasts, macrophages, vascular epithelium, and lymphocytes that invade tumors. The normal cells from other body areas and outside the tumor mass also start to "behave" improperly under the influence of the tumor microenvironment and cancer cells. These cells may not have the same gene alterations as CSCs to become a member of that specific malignancy. Regular treatment protocols such as radiation and chemotherapy remain ineffective for tumors developed due to CSCs. Consequently, targeting CSCs, which are believed to drive tumor growth and recurrence, may be a promising strategy for anticancer treatment (Ajmeera and Ajumeera 2024). The major obstacles to the development of chemotherapeutic drugs are non-selective tissue distribution, cross-resistance to a range of drugs, significant adverse effects, poor physicochemical properties, and a narrow therapeutic index where the therapeutic and toxic doses are close (Kifle et al. 2021). As a result, most anticancer drugs fail in clinical trials. The optimal strategy for cancer chemotherapy is to selectively target cancer cells, reducing exposure to healthy tissues and minimizing side effects. However, less than 5% of the licensed pharmaceuticals have been designed for selective distribution to tumor locations while limiting severe adverse effects (Bae and Park 2011).

It is essential to concentrate on more recent and target-oriented therapy that destroys cancer cells with fewer or no side effects in order to lower the hazards connected with present treatment approaches. The development of drug resistance by tumor cells leading to a reduction in the anticancer activity of the therapeutic drugs is another challenge (Ajmeera and Ajumeera 2024). Based on estimates, around 90% of approved non-cancer agents, spanning various therapeutic classes, exhibit positive effects that can be explored for alternative uses, providing a cost-effective strategy for cancer treatment through drug repurposing (Rodrigues et al. 2022).

Repurposed Drugs for Cancer Targets and Its Current Scenario

Untoward side effects of traditional medications and drug resistance are significant challenges in cancer treatment. It often reduces the efficacy of standard therapies and hence demands an urgent need for innovative approaches. Repurposing existing drugs can help to address the genetic heterogeneity of cancer and support personalized medicine. Since the pharmacodynamic and pharmacokinetic profiles of many repurposed drugs are previously established, they often reduce research and development costs. Finding the reasons causing cancer, prevention, recovery, palliative care, and socio-economic burdens are other aspects. In order to solve all these challenges, oncology drug discovery programs focus on different kinds of safer and newer pharmaceutical drugs (Weth et al. 2024). Medications or other compounds occasionally referred to as "molecularly targeted drugs" are considered targeted cancer therapy. The way such medications work is by interfering with growth molecules, which prevents cancer from growing and spreading (Debela et al. 2021). Table 1 highlights how traditional drugs have been repurposed to target specific cancer pathways, focusing on improving clinical outcomes while addressing the side effects and limitations of conventional therapies. The clinical status and ongoing research into these drugs are key points of interest, underscoring the potential of drug repurposing in oncology.

In drug discovery and pharmacological studies, drug effectiveness is commonly measured by IC50 values, indicating the concentration necessary to limit a biological process by 50%. The efficacy of repurposed drugs is widely regarded as the key factor influencing the success of pharmaceutical products, as it reflects how well a treatment achieves its intended purpose. Table 2 provides further details on selected potential repurposed drugs, including their chemical structures, biological targets (mechanisms of action), and biological activities regarding IC50 values for both their traditional and new anticancer indications, highlighting their market potential.

Important Cancer Targets Involved in the Development of Repurposed Drugs

a. Cyclooxygenase (COX) 1/2

Cyclooxygenase (COX) is the membrane-bound enzyme that plays a significant role in the biosynthesis of prostaglandins (PGs), such as PGE2, PGF2α and thromboxane (TX). These biologicals are useful in the process of inflammation. COX-1 and COX-2 are the two isoforms of the COX enzyme. In many healthy tissues, the COX-1 isoform is constitutively expressed and maintains steady-state prostaglandin levels for "housekeeping functions". Conversely, COX-2 is an inducible isoform that is stimulated by substances like prostaglandins, which is generally either absent or expressed at a low level in organs and tissues that are normal, but it is overexpressed in many tissues associated with tumors and inflammation. In anticancer therapy, as in developing NSAIDs (non-steroidal anti-inflammatory drugs), molecules with

Table 1. Molecular targets and treatments associated with repurposed drugs for cancer ⏎

Targets/ Pathways	Drugs with Traditional Medical Indications (Anticancer)	Reported Side Effects Due to Traditional Medication	Repurposed Drugs	Category	Repurposed Drugs with New Medical Indications	Clinical Status	References
Inhibition of over-expressed **COX1/2 enzymes** (refer to Figure 3 for mechanism of action)	Licofelone	Risk of myocardial infraction and stroke	Aspirin	NSAID	Prostate and colorectal cancer	Phase IV	(Joshi et al. 2021)
			Diclofenac		Colorectal, breast, pancreatic, ovarian, and prostate cancer	Phase II	(Pantziarka et al. 2016)
			Ibuprofen		Gastric, lung, breast, and liver cancer	Phase IV	(Shen et al. 2020)
			Celecoxib		Colorectal, hepatic, and breast cancer	Phase III/ IV	(Huang et al. 2017)
			Indomethacin		Colorectal cancer	Phase I	(Hull 2003 et al.)
Inhibition of the **mTOR** signaling pathway (refer to Figure 4 for mechanism of action)	Everolimus Perifosine	High blood pressure, blurred vision, and blood clots may cause belly pain, hand-foot syndrome, and kidney damage may cause swelling;	Itraconazole	Antifungal	Umbilical vein endothelial cell cancer and endometrial carcinoma	Phase II	(Tsubamoto et al. 2017)
			Thiazolidinedione	Antidiabetic	Prostate cancer	Phase II	(Blanquicett et al. 2008)

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Targets/ Pathways	Drugs with Traditional Medical Indications (Anticancer)	Reported Side Effects Due to Traditional Medication	Repurposed Drugs	Category	Repurposed Drugs with New Medical Indications	Clinical Status	References
		development of new cancer resulting from treatment of a prior cancer; musculoskeletal pain, thrombocytopenia, neutropenia, anemia, hyponatremia, pneumonia, ring-shaped perilimbal corneal ulceration	Metformin	Antidiabetic	Breast, prostate, lung, endometrial, pancreas, ovarian cancer and osteosarcoma	Phase IV	(Zhao et al. 2019)
			Rapamycin	Immunosuppressant	Renal cancer	Approved	(Luan et al. 2003)
Alteration of **JAK-STAT pathway** (refer to Figure 5 for mechanism of action)	Ruxolitinib	Thrombocytopenia, bladder pain, blood in the urine or stools, bruising, chest tightness, cloudy urine, and collection of blood under the skin	Chloroquine	Antimalarial	Skin, triple-negative breast cancer	Phase III	(Choi et al. 2014)
			Peficitinib	Antirheumatic	Epithelial ovarian cancer	-	(Standing et al. 2023)
			Quercetin	Antiinflammatory	Prostate, cervical, breast, lung, colon, liver cancer	Phase III	(Luo et al. 2016)
Inhibits **overexpressed EGFR**	Osimertinib, Gefitinib, Lapatinib,	Light-headedness, fainting, vision changes, irregular	Leflunomide	Antirheumatic	Breast and prostate cancer	Phase III	(Huang et al. 2015)

(refer to Figure 6 for mechanism of action)	Afatinib, Dacomitinib	heartbeat, discomfort in the groin, chest, or legs, particularly the calves, arm or leg pain, redness, or swelling, speech slurred, abrupt lack of coordination, excruciating headache, arm or leg weakness or numbness, breathing	Brexpiprazole	Antipsychotic	Pancreatic, non-small cell lung cancer	-	(Sanomachi et al. 2019)
			Doxazocin	Antihypertensive	Prostate and bladder cancer	-	(Batty et al. 2016)
Inhibition of the **Wnt/ β-catenin pathway** (refer to Figure 7 for mechanism of action)	Sorafenib	Mitochondrial dysfunction causes energy metabolism disorders, muscle damage, myocardial ischemia, congestive heart failure, bleeding gums, blood in the urine or stools, blood in cough, and difficulty in breathing or swallowing	Artemisinin	Anti-malarial	Lung, colon, breast, ovarian, and pancreatic cancer	Phase II	(Hui et al. 2016)
			Mesalazine	Anti-inflammatory	Colorectal cancer	Phase II	(Stolfi et al. 2012)
			Nigericin	Antibiotic	Ovarian, breast, and colorectal cancer	-	(Wang et al. 2017)
Inhibits **PI3K target**	Idelalisib Alpelisib	Steven-Johnson syndrome, toxic epidermal necrolysis,	Naproxen	NSAID	Breast, colon, bladder cancer and osteosarcoma	Phase IV	(Kim et al. 2014)

(*Contd*.)

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Targets/ Pathways	**Drugs with Traditional Medical Indications (Anticancer)**	**Reported Side Effects Due to Traditional Medication**	**Repurposed Drugs**	**Category**	**Repurposed Drugs with New Medical Indications**	**Clinical Status**	**References**
		pneumonia, sepsis, reduced appetite, high blood sugar levels, severe cutaneous adverse reactions, embryo-fetal toxicity	Curcumin	Antioxidant, anti-inflammatory	Multiple myeloma, breast, colon, and prostate cancer	Phase III	(Hamzehzadeh et al. 2018)
Inhibits **NF-kB**	Thalidomide	Dry skin, bleeding gums, double vision, seizures, chest pain, xerostomia, orthostatic hypertension	Niclosamide	Anthelminthic	Colorectal, breast, ovarian cancer	Phase II	(Nazam et al. 2023)
Notch Signalling Pathway	Cisplatin	Alopecia, loss of taste, dry mouth	Lomitapide	Antihyperlipidemic	Colorectal cancer	-	(Czerwonka et al. 2023)
Gαq coupled HTR2B Reduces Proto-oncogene tyrosine-protein kinase Src	Dasatinib, Bosutinib	Thrombocytopenia, anemia, pyrexia, liver abnormalities	Aripiprazole	Anti-psychotic	Colon, gastric cancer	-	(Liu et al. 2023)

H2 receptor antagonist	-	-	Cimetidine	Gastric and peptic ulcer	Breast, cervical, ovarian, colorectal, prostate, and gastric cancer	Phase III	(Pantziarka et al. 2014)
ALDH, NAD+ dependent proteins	Bufalin	Anaemia	Disulfiram	Antialcoholism	Breast cancer	Phase II	(Han et al. 2015)
Inhibits **DNA methylation**	Azacitidine Decitabine	Breathing difficulties, facial swelling or redness, thrombocytopenia	Hydralazine	Anti-hypertensive	Metastatic cervical, ovary, breast cancer	Phase III	(Ruiz et al. 2016)
Targeting **AMPK**	Methotrexate Pemetrexed	Cough and breathlessness, alopecia.	Phenformin	Diabetic	Melanoma	-	(Petti et al. 2012)
P53, FACT-CK2-p53 axis	Vismodegib, Sonidegib, Glasdegib	Myopathy, muscle spasms, pain, tenderness, alopecia, hepatotoxicity	Quinacrine	Rheumatic disease	Breast cancer	Phase II	(Preet et al. 2012)
Estrogen Receptor	Tamoxifen	Increased risk of stroke, endometrial cancer, cataracts, uterine sarcoma, blood clots in the lungs and legs, lung inflammation, low platelet and high calcium levels	Raloxifene	Osteoporosis	Invasive breast cancer	Phase III	(Vogel 2011)

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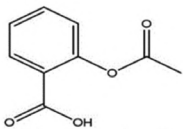
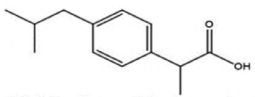
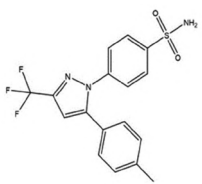
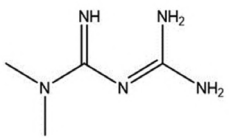
Targets/ Pathways	**Drugs with Traditional Medical Indications (Anticancer)**	**Reported Side Effects Due to Traditional Medication**	**Repurposed Drugs**	**Category**	**Repurposed Drugs with New Medical Indications**	**Clinical Status**	**References**
Target **HIF-1**	Doxorubicin, Daunorubicin	Alopecia, back pain, headache, tiredness	Digitoxin	Cardiovascular drug	Pulmonary, prostate, and breast cancer	Phase II	(Kulkarni et al. 2017)
Enhancer of **EZH2**	Tazemetostat	Decreased appetite, alopecia	Ribavirin	Antiviral	Nasopharyngeal carcinoma and glioblastoma	Phase IV	(Huq et al. 2020)
Inhibits **ARP**	Darolutamide, Apalutamide	Ischemic heart disease and angina, increased risk of broken bones, thinning bones, and fractures	Ketoconazole	Anti-viral	Prostate, melanoma, breast, hepatocellular carcinoma	Phase IV	(Attard et al. 2011)

Table 2. Clinical efficacy of repurposed drugs in advanced clinical stages (Phase III/IV) of trials

Chemical Structure of Repurposed Drug	Anticancer Action of Repurposed Drugs	Reported IC_{50} of Traditional Indications (μM)	Reported IC_{50} of Anticancer Treatment (μM)	References
Aspirin 	Induces tumor cell apoptosis and interferes in cell signaling	COX1 - 44.40 COX2 - 1110.1	COX1 - 2.8 COX2 - 26	(Mitchell et al. 1993; Lloyd et al. 2003)
Ibuprofen 	Reduce tumor cell proliferation	COX1 - 12 COX2 - 80	Lung cancer - 3000 Breast cancer - 1800 Liver cancer - 1200	(Kato et al. 2010; Shen et al. 2020)
Celecoxib 	Regulates proliferation of tumor cells, migration, and invasion	COX1 - 82 COX2 - 6.8	Breast cancer - 40.05 Colorectal cancer - 50, Anti-proliferative properties- COX-1 - 0.34 COX-2 - 2.25	(Kato et al. 2010; Gurpinar et al. 2014)
Metformin 	Inhibits CSCs self-renewal ability	DPP4 - 56.90	Breast cancer - 14020 Ovarian cancer - 20000	(Lindsay et al. 2005; Jiang et al. 2022)

(*Contd*.)

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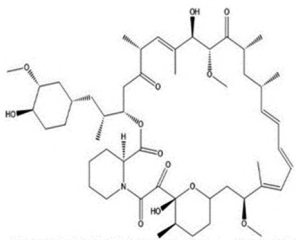
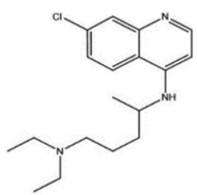
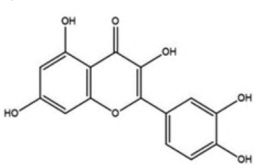
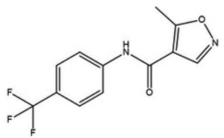
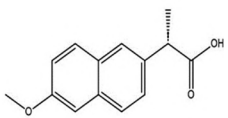
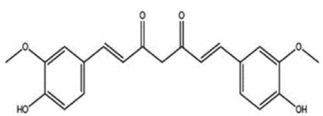
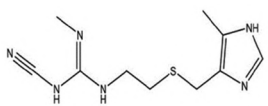
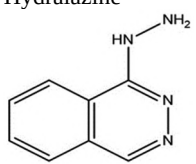
Chemical Structure of Repurposed Drug	**Anticancer Action of Repurposed Drugs**	**Reported IC_{50} of Traditional Indications (μM)**	**Reported IC_{50} of Anticancer Treatment (μM)**	**References**
Rapamycin 	Reduce proliferation, induces apoptosis of tumor cells, and suppresses angiogenesis of tumor	mTORC1 - 0.0000633 mTORC2 - 0.0005349	0.00019	(Schreiber et al. 2019; Grabiner et al. 2014)
Chloroquine 	Induces apoptosis of tumor cells by inhibiting autophagy	Heme polymerase - 0.0002 to 0.2834	91.48	(Randrianarivelojosia et al. 2002; Liu et al. 2024)
Quercetin 	Inhibiting tumor proliferation, invasion, and metastasis	DPPH - 6.08 ABTS - 1.68	10 to 100	(Tian et al. 2021; Asgharian et al. 2022)

Leflunomide 	Slows down the proliferation of tumor cell	DHODH - 12.5	31.36	(Sanders and Harisdangkul 2002; Huang et al. 2015)
Naproxen 	Causes apoptosis and G1 cycle arrest in tumor cells	COX1 - 35.48 COX2 - 64.62	5 to 100	(Hinz et al. 2008; Birgul et al. 2022)
Curcumin 	Stimulates CSCs	DPPH - 32.86	37.36 ±1.88	(Ernala et al. 2014; Kostrzewa et al. 2021)
Cimetidine 	Reduce tumor cell proliferation and suppress angiogenesis	H2R - 1.6	Gastric carcinoma-196.56 Colorectal carcinoma - 903.54	(Pounder et al. 1976; Sieger et al. 1999)
Hydralazine 	Induces apoptosis and causes DNA damage	NA - 2.49 KCh - 4370.19	165.1	(Criscione et al. 1986; Lafi et al. 2023)

(*Contd*.)

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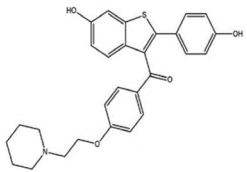
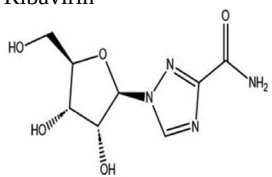
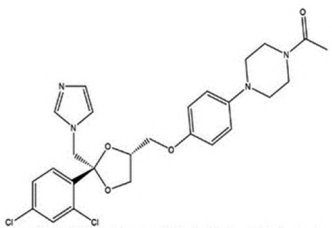
Chemical Structure of Repurposed Drug	**Anticancer Action of Repurposed Drugs**	**Reported IC_{50} of Traditional Indications (μM)**	**Reported IC_{50} of Anticancer Treatment (μM)**	**References**
Raloxifene 	Inhibits tumor cell proliferation	ERα - 0.0024-0.004 ERβ - 0.043	0.0002	(Gennari et al. 2007; Powell et al. 2020)
Ribavirin 	Decrease cell proliferation impairs migration, invasion, and adhesion	RdRp - 2.65	53.6	(Mayor et al. 2021; Ochiai et al. 2020)
Ketoconazole 	Induces tumor cell cycle arrest	LDM - 0.24	35.1	(Back and Tjia 1991; Bae et al. 2018)

greater COX-2 selectivity are preferred, as COX-1 inhibition is less desirable due to gastrointestinal side effects. COX enzymes convert arachidonic acid (AA) to the endogenous peroxidation intermediate PGH2, which is subsequently modified by prostaglandin synthase to produce five structurally related prostaglandins, such as PGD2, PGE2, PGI2, PGF2α, and TXA2. The two vital enzymes that facilitate the beginning and terminal phases of the prostaglandin production pathway are COX-2 and prostaglandin E2 synthase (PGES). The COX-2-PGE2 signaling pathway functions in several capacities: establishing a tumor-supportive microenvironment, modulating tumor immune surveillance, altering DNA methylation mechanisms, and inhibiting tumor epithelial cell survival (Mohsin et al. 2022). PGE2 exerts its effects via binding to four G-protein-coupled receptors (GPCRs), namely the E-type prostanoid receptors (EP1–EP4). Each EP receptor couples to distinct signaling pathways. PGE2 is metabolized to its inactive form, 15-keto-PGE2 (PGEM), via 15-hydroxyprostaglandin-dehydrogenase (15-PGDH) (Jin et al. 2023). The detailed COX 1/2 inhibition pathway is illustrated in Figure 3.

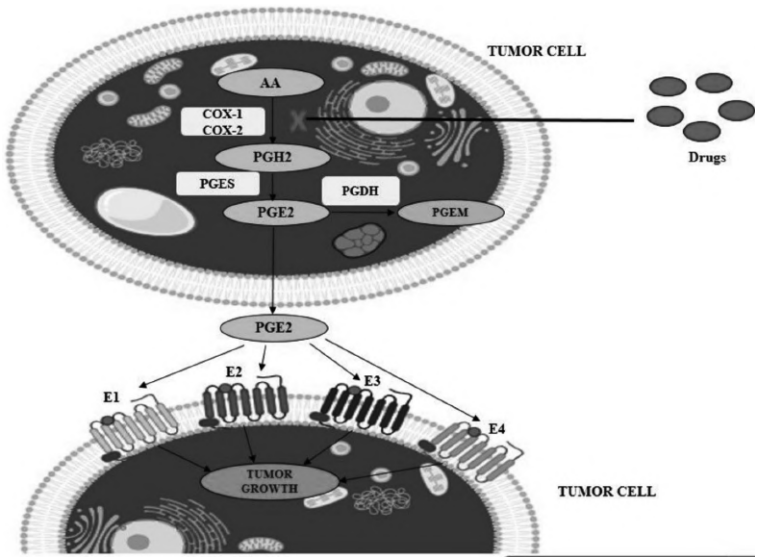


Figure 3. COX inhibition pathway. ⏎

Aspirin has been shown to inhibit COX-2 activity by acetylation, which decreases the synthesis of proinflammatory prostaglandins and raises AA levels, potentially triggering apoptosis (Joshi et al. 2021). Aspirin also affects platelet function, which is involved in cancer progression and metastasis. COX-2 is overexpressed in colon cancer, and activated platelets may contribute to this overexpression in colorectal cancers. COX-1/2 inhibition by NSAIDs like diclofenac (Pantziarka et al. 2016), ibuprofen (Shen et al. 2020), celecoxib (Huang et al. 2017), and indomethacin (Hull et al. 2003) inhibits cancer. COX-2 has become a viable target for novel anticancer medications, as it reduces PGE2, a type of prostaglandin that contributes to the proliferation and cycle progression of cells.

b. Mammalian Target of Rapamycin Pathway

The mammalian target of rapamycin (mTOR) is a protein kinase enzyme that belongs to the phosphatidylinositol 3-kinase (PI3K) family and plays a crucial role in cell proliferation, autophagy, and apoptosis. It is often activated in malignancies, affecting the transcription of genes, synthesis of proteins, and tumor metabolism (Saxton and Sabatini 2017). mTOR forms two distinct complexes with protein: mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). Overactivation of mTORC1 can encourage the growth of tumors. Meanwhile, mTORC2 controls the expression of mTORC1 via PI3K/Tuberous sclerosis complex 1 and 2 (TSC)/Ras homolog enriched in the brain (RHEB) pathway.

Furthermore, mTOR promotes the growth of tumors via the following six pathways. Pathway 1 involves activation of mTORC1 through extracellular growth signals and intracellular liver kinase B1 mutations. These mutations are responsible for the decrease of ubiquitination of histone H2A, which leads to impaired DNA repair and promotes tumor growth (Zou et al. 2020). Pathways 2 and 3 regulate mTORC1 activity by RHEB, TSC2, Tumor necrosis factor receptor-associated factor 2 (TRAF2), and Otubains deubiquitinase 7B (OTUD7B). These factors regulate mTORC1 or mTORC2 activities either by enhancing mTORC1 and/or by inhibiting mTORC2. Mutated Ras interacts with mTOR and mitogen-activated protein kinase-associated protein 1 (MAPKAP1) via pathway 4 by promoting mTORC2 expression. OTUD7B homolog 1 (B7-H1) is upregulated by deleting the phosphate and tensin homology on chromosome ten (PTEN) gene, enhancing tumor development and invasion through pathways 5. Pathway 6 restricts the accumulation of reactive oxygen species and DNA damage in colitis cancer, hence contributing to the invasion and metastasis of liver cancer through the upregulation of matrix metalloproteinase 9 (MMP-9). The detailed relationship between mTOR pathways and tumor genesis is illustrated in Figure 4.

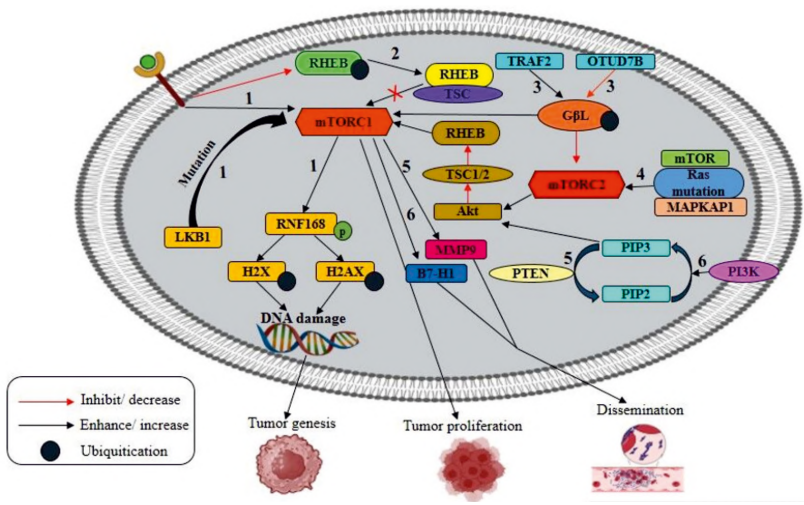


Figure 4. Relationship between mTOR pathway and tumors.

Repurposed anticancer drugs like itraconazole, thiazolidinedione, metformin, and rapamycin have been associated with reduced cancer risk. The clinically utilized itraconazole antifungal medication also exhibits strong antiangiogenic and anticancer properties (Tsubamoto et al. 2017). It inhibits the mTOR signaling pathway, which is a critical regulator of the endothelial cell function and angiogenesis process. Likewise, thiazolidinedione controls insulin signaling in tissues like muscle and fat, inhibiting insulin-like growth factor 1 and insulin-induced cell proliferation. Metformin, which is approved by the FDA for the treatment of type 2 diabetes, has been shown to lower elevated insulin levels, which, in turn, activates the PI3K-mTOR signaling pathway, inhibiting the proliferation of tumors that express insulin receptors (Zhao et al. 2019). Rapamycin is an immunosuppressant that has been approved for preventing kidney transplant rejection. It acts by allosterically inhibiting mTORC1 (through TRAF2 and OTUD7B activity), which is actively involved in the development of tumors with limited influence on the activation of mTORC2 (Luan et al. 2003).

c. JAK/STAT Pathway

The JAK/STAT signal transduction system uses Janus kinase (JAK) to transfer information from the extracellular environment to the intracellular environment by phosphorylating transcription factors STATs (Signal transducer and activator of transcription). Under normal circumstances, this JAK/STAT pathway stimulates the release of several growth factors, cytokines, interleukins, and hormones, which increases cell migration, proliferation, differentiation, and cell death. However, this pathway also plays an important role in the development of a highly lethal form of skin cancer (melanoma). The JAK family comprises four essential membrane-associated JAKs, which are non-receptor tyrosine kinases that contribute to this signaling process (Hu et al. 2021; Rah et al. 2022).

When two neighboring JAKs are brought close to one another by ligand-mediated receptor multimerization, the JAK-STAT pathway is triggered, facilitating trans-phosphorylation. JAKs phosphorylate several substrates, encompassing significant non-receptor substrates, receptors, and STATs. The seven latent transcription factors that comprise the STAT family (STAT1 to STAT7) normally shuttle between the cytoplasm and the nucleus in response to extracellular stimuli or abnormal activation of other signaling processes. It has been discovered that STAT3-induced gene expression is linked to angiogenesis, metastasis, apoptotic resistance, and immune response inhibition. Figure 5 demonstrates the detailed mechanism of the JAK/STAT inhibition pathway. Phosphorylated STAT1 to STAT3 ratios may be a biomarker for melanoma development. Repurposed drugs such as chloroquine (Choi et al. 2014), peficitinib (Standing et al. 2023), and quercitin (Luo et al. 2016) have been associated with a reduced risk of melanoma, triple-negative breast cancer, and cancers of the epithelium of ovary, lung, prostate, cervix and their recurrence via JAK/STAT inhibition. These drugs have shown promise in lowering the possibility of cancers and their reemergence via JAK/STAT inhibition.

d. Epidermal Growth Factor Receptor (EGFR)

The epidermal growth factor receptor (EGFR) is a cellular protein associated

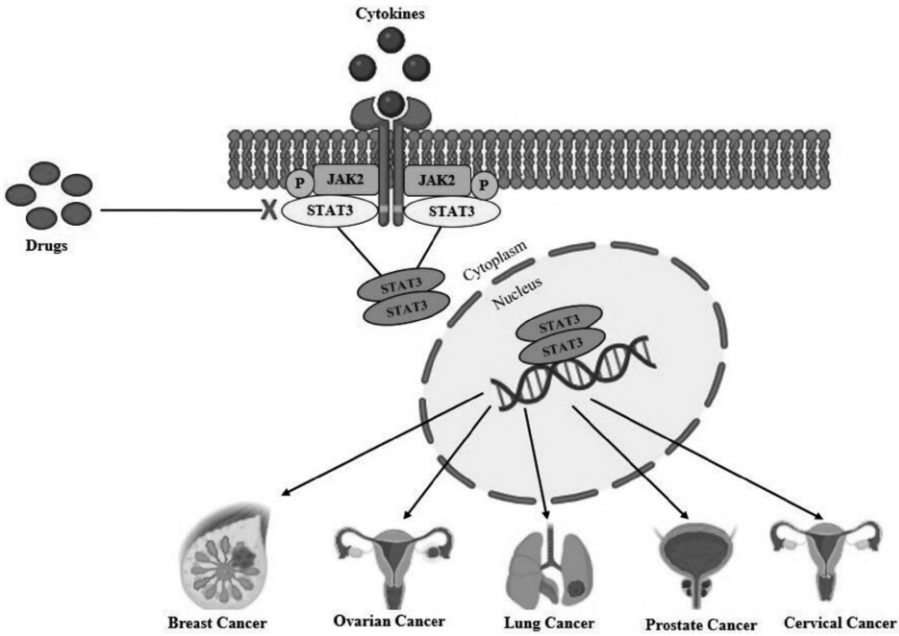


Figure 5. JAK/STAT inhibition pathway. ⏎

with commonly altered genes in human cancers, characterized by overexpression, amplification, and mutation. Inhibiting EGFR activity may disrupt the signal transduction pathways that control tumor cell growth, proliferation, and resistance to apoptosis (Wykosky et al. 2011).

The proto-oncogene c-erbB1 encodes EGFR, and the transmembrane protein is divided into three sections: the extracellular domain, receptor tyrosine kinase (RTK) active intracellular domain, and the hydrophobic α-helix region of the single-transmembrane domain. When a signaling molecule attaches itself to the extracellular domain of the receptor, it forms a dimer on the membrane that phosphorylates and activates protein kinases to create signaling complexes. Tumor cell proliferation, differentiation, and migration are promoted by phosphorylated sites because they serve as binding sites for additional downstream signaling molecules and activate the downstream signaling pathways such as RAS-RAF-MEK-ERK, PI3K-AKT-NF-kB/mTOR, and JAK-STAT, as shown in Figure 6 (Peng et al. 2023).

Repurposed EGFR inhibitors like leflunomide, brexpiprazole, and doxazocin have been used to target EGFR in various cancers. Leflunomide inhibits pyrimidine synthesis, disrupts the cell cycle, and has antiproliferative activity in melanoma, chronic lymphocytic leukemia cells, and human medullary thyroid carcinoma (Huang et al. 2015). Brexpiprazole inhibits the signal transduction of the EGFR pathway, sensitizing cells to the targeted drug (Sanomachi et al. 2019). Lastly, doxazosin stops the growth of cells and triggers the death of cells by blocking the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and mitogen-activated protein kinase (MAPK) signaling pathways (Batty et al. 2016).

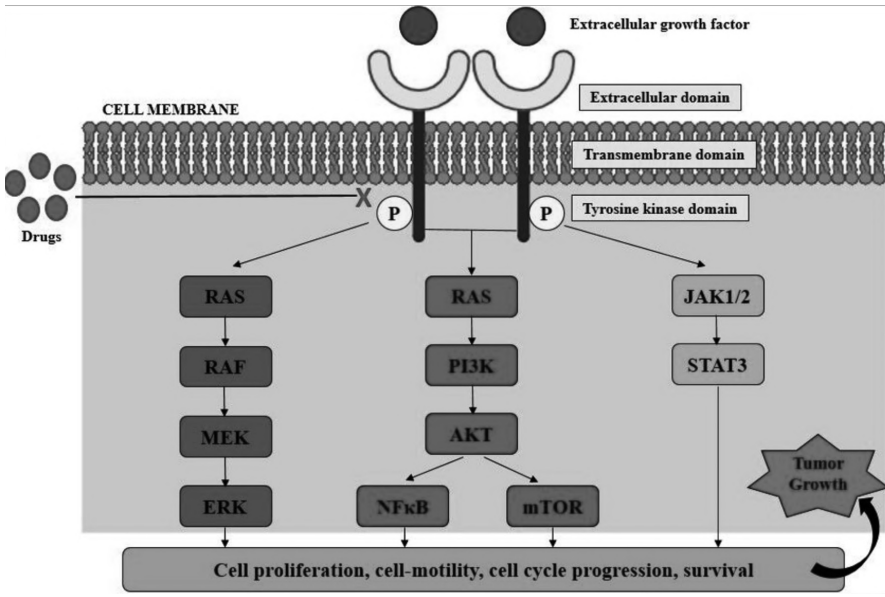


Figure 6. EGFR inhibition pathway.

e. Wnt/β-Catenin Pathway

The Wnt signaling cascade is essential for maintaining homeostasis and regulating biological processes, such as stem cell renewal, apoptosis, migration, differentiation, and genetic stability. This pathway comprises more than 12 receptors, co-receptors, and 19 Wnt ligands (Zhao et al. 2024). There are two types of Wnt signaling pathways: canonical and non-canonical. In the canonical Wnt signaling pathway, Wnt ligands are absent. The glycogen synthase kinase 3β (GSK3β) uses the frizzled (FZD) lung resistance-related protein (LRP5/6) receptor complex to phosphorylate β-catenin, which results in β-catenin breakdown and prevents β-catenin translocation from the cytoplasm to the nucleus. Further, canonical Wnt signaling β-catenin activates the gene transcription through binding with T cell factor/lymphoid enhancer factor (TCF/LEF). It enhances the presence of histone-modifying co-activators and thus initiates the transcriptional expression of downstream target genes. In the non-canonical Wnt signaling pathway, Wnt binds with FZD receptors and LRP co-receptors. Thereby, it triggers phosphorylation of LRP receptors by GSK3β, resulting in the release of β-Catenin from the axin complex, allowing its entry into the nucleus. Non-canonical Wnt pathways may also be involved in cancer progression when activated or inhibited abnormally (Ajmeera and Ajumeera 2024). The difference between canonical and non-canonical Wnt pathways is elucidated in Figure 7.

Artemisinin, mesalazine, and nigericin have been used for cancer treatment in various diseases. Artemisinin suppressed lung tumorigenesis and inhibited tumor metastasis, making it an excellent chemopreventive drug with significant anticancer efficacy (Hui et al. 2016). Mesalazine inhibited the Wnt/β-catenin pathway by inhibiting protein phosphatase 2A, a key signaling pathway in CSCs (Stolfi et al.

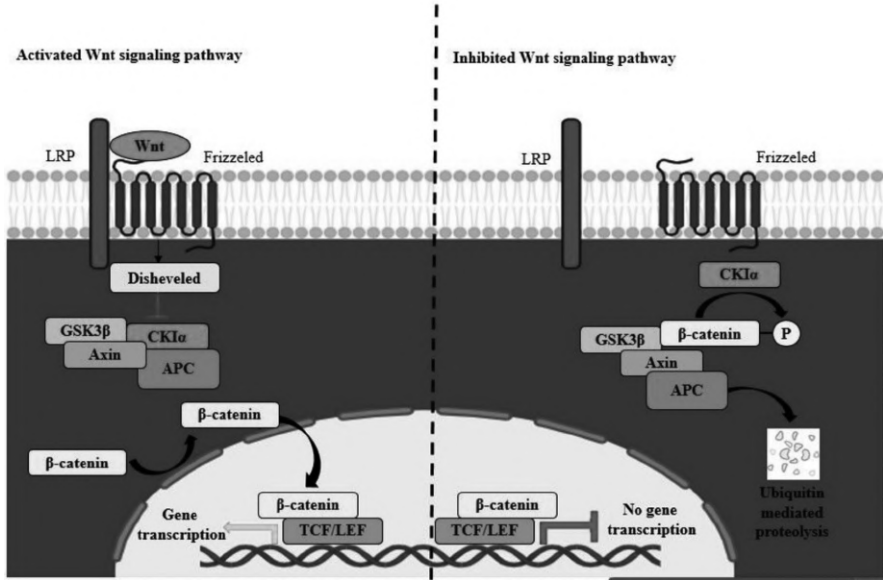


Figure 7. Wnt/β-catenin pathway in the presence and absence of Wnt ligands. ⏎

2012). Nigericin attenuated the potential for colorectal cancer to cause tumors by downregulating Wnt-related and target proteins (Wang et al. 2017). It is also inhibited by focusing on the β-catenin degradation complex directly. These findings validate the role of β-catenin in the Wnt/β-catenin pathway as a crucial mediator and participant.

Pharmaceutical Challenges and Opportunities in Drug Repurposing for Cancer

Drug repurposing, the approach of finding new therapeutic uses for existing drugs, has gained importance in cancer research. While it offers advantages such as shorter drug discovery timelines and reduced costs, it also presents several pharmaceutical challenges. A primary concern is the lack of target specificity, as many drugs repurposed for cancer were originally designed for other conditions. Hence, achieving therapeutic effects for such drugs may require higher doses, thereby altering the drug interactions and potentially introducing new mechanisms of action and off-target effects. Such changes can lead to unexpected side effects, especially when proper consideration of dosage and serum concentration is overlooked.

Repurposed drugs frequently result in undesirable side effects due to inadequate pharmacokinetics and low bioavailability for the new target, which poses significant challenges for their entry into the market. Certain repurposed drugs may exhibit restricted distribution among tumor tissues due to inadequate solubility, necessitating preformulation studies and stability protocols to achieve therapeutic concentrations. Hence, it is essential to meticulously examine the pharmacodynamics and

pharmacokinetics of repurposed medications to ascertain appropriate doses, dosing intervals, and drug interactions. Moreover, repurposed drugs may encounter the same challenges faced in traditional drug development, including potential failure during clinical trials attributable to a limited number of volunteers and variability among them. Regulatory hurdles further complicate the process, as even though these drugs have undergone prior safety evaluations, gaining approval for new cancer indications requires additional clinical trials, which can be both lengthy and costly (Natsheh et al. 2024; Xia et al. 2024).

Cancer cells display the ability to develop resistance to treatments, which also limits the long-term success of repurposed drugs, making it crucial to address these resistance mechanisms. Furthermore, intellectual property and commercial viability are significant obstacles, as many repurposed drugs are off-patent. This can discourage pharmaceutical companies from investing in the necessary trials and commercialization due to the lack of financial incentives. Collectively, these challenges make it difficult to exploit drug repurposing fully as a strategy in cancer treatment.

Nevertheless, despite these hurdles, drug repurposing remains a valuable and cost-effective strategy for improving cancer therapy outcomes, provided these challenges are carefully managed.

Future Prospects of Repurposing in Cancer Chemotherapy

Cancer remains a primary cause of death globally, with breast and colon cancer among the most common types. The tumor cells' development of resistance limits the effectiveness of current treatments, underscoring the need for new therapeutic strategies. Approximately 20 million people had a cancer diagnosis in 2023, highlighting the urgent need for new treatment approaches as current therapies continue to face challenges. The growing incidence of cancer, coupled with the limitations in available care in many regions, calls for innovative strategies like drug repurposing (Pushpakom 2022). Repurposing existing drugs, especially generics, offers a cost-effective alternative, particularly for low-income countries where the burden of cancer is substantial. By leveraging the known biological activities of a drug, researchers can investigate its potential for new therapeutic targets while considering off-target effects and ensuring the safety of the drug at higher doses (Pfab et al. 2021).

Despite the promise of repurposing, few non-cancer medications have been authorized for use in the treatment of cancer, though many are undergoing preclinical and clinical trials. To fully realize the possibilities of this strategy, more efforts are needed to address regulatory hurdles and patent challenges.

Abbreviations

ABTS:	2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid
ALDH:	Aldehyde dehydrogenase
ARP:	Actin related protein

DHODH:	Dihydroorotate dehydrogenase
DNA:	Deoxyribonucleic acid
DPP4:	Dipeptidylpeptidase
DPPH:	2,2-diphenyl-1-picrylhydrazyl
ERα:	Estrogen receptor alpha
ERβ:	Estrogen receptor beta
FACT-CK2 axis:	Casein kinase 2
FDA:	Food and Drug Administration
HIF-1:	Hypoxia-inducible factor 1
H2R:	Histamine 2 receptor
HTR2B:	5-Hydroxytryptamine receptor 2B
KCh:	Potassium channel
LDM:	Lanosterol 14-demethylase
NA:	Nor-adrenaline
NAD+:	Nicotinamide adenine dinucleotide
P53:	Protein 53
PK:	Pharmacokinetics
R&D:	Research and development
RdRp:	RNA-dependent RNA polymerase
USFDA:	United States Food and Drug Administration
WHO:	World Health Organization

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CHAPTER
5

Anticancer Drugs for Repurposing Against Gram-Negative and Gram-Positive Bacterial Infections

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Introduction

Gram-negative and Gram-positive bacteria are a significant concern in healthcare settings due to their ability to cause a wide range of infections, such as pneumonia, bloodstream infections, wound or surgical site infections, and meningitis (Morris and Cerceo 2020), which are often difficult to treat. Bacterial infections, especially hospital-acquired ones, pose a significant burden on healthcare systems worldwide, with reported costs of $136 million per year (Balasubramanian et al. 2023), requiring ongoing efforts in surveillance, infection prevention and control, antibiotic stewardship, and research into new treatment options to mitigate their impact.

One of the main difficulties in tackling bacteria is their high efficiency in acquiring antimicrobial resistance (AMR) encoded by genomic, transcriptomic, and proteomic changes (Yelin and Kishony 2018). Compounding the problem of AMR, with 4.95 million deaths associated with bacterial AMR and 1.27 million deaths attributable to bacterial AMR, is the immediate threat of a reduction in the discovery and development of new antibiotics (Antimicrobial Resistance Collaborators 2022). The World Health Organization (WHO) (Tacconelli et al. 2018) and other European institutions have recently underscored the dangers posed by these infections (O'Neil 2016; Petersen et al. 2023). The situation is particularly concerning since resistance to colistin, fluoroquinolones, and β-lactam (i.e., carbapenems, cephalosporins, and

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penicillins) and macrolides – antibiotics often considered first line for empirical therapy of severe infections – accounts for more than 70% of deaths attributable to AMR across pathogen (Antimicrobial Resistance Collaborators 2022). Practical solutions are urgently needed, as stressed by various stakeholders. New policies and actions are necessary to prevent the projected figures for 2050, which estimate ten million deaths worldwide due to antimicrobial resistance (O'Neil 2016). Especially troublesome are cases of pathogens like *Acinetobacter baumannii* and *Escherichia coli* for which continuous increase in AMR is reported, which also account for most associated diseases and deaths (ECDC and WHO 2023).

A literature review was conducted to search for potential non-antimicrobial candidate drugs that are not intentionally used as antimicrobial agents but have one or more antimicrobial properties. A variety of drug families have been considered, including anthelmintics, anti-inflammatory/immunomodulatory drugs, antipsychotic and antidepressant drugs, statins, iron-storage drugs, and anticancer drugs (Miro Canturri et al. 2019; Miro Canturri and Smani 2022). While these drugs are approved for clinical indications, promising antibacterial activities have been reported in preclinical and clinical studies. It is noteworthy that repurposing drugs is a promising approach with several advantages. As drugs are approved by the Federal Drug Administration (FDA), information about their pharmacological characteristics (both safety and pharmacokinetic) in preclinical and clinical trials is widely available. Therefore, the time and economic costs associated with repurposing these drugs for other therapeutic applications, such as treating bacterial and fungal infections, will be minimized.

In this chapter, we focus on the current state of knowledge regarding the repurposing of anticancer drugs in terms of their antimicrobial efficacy, modes of action, and breadth of the spectrum against Gram-negative and Gram-positive bacteria, as well as the advances to date in their development as antimicrobial agents for clinical use.

The Potential of Anticancer Drugs for Treatments of Bacterial Infections

The antibacterial activity of several anticancer drugs has been reported *in vitro* and *in vivo* in non-vertebrate and vertebrate models against Gram-negative and Gram-positive bacteria.

Gram-Negative Bacteria

Gallium Nitrate

Gallium nitrate, an anticancer drug for the treatment of lymphoma and bladder cancer, is one of the most studied and advanced cancer drugs in clinical development with promising data. Gallium nitrate has demonstrated an inhibitory effect on bacterial growth in *P. aeruginosa* at concentrations >3.13 μM *in vitro* (Kaneko et al. 2007; Frangipani et al. 2014); although the presence of pyoverdine and proteases in human serum reduces the efficacy of gallium nitrate against *P. aeruginosa* (Bonchi et al.

2015). At non-bactericidal concentrations, gallium nitrate can affect the production of virulence factors of *P. aeruginosa* (Kaneko et al. 2007; García-Contreras et al. 2014). In murine models of acute and chronic lung infections by *P. aeruginosa*, gallium nitrate has reduced lung injury and bacterial loads in the tissues of animals (Kaneko et al. 2007).

Against *A. baumannii*, gallium nitrate has demonstrated an inhibitory effect on bacterial growth in a collection of 58 multidrug-resistant (MDR) clinical isolates *in vitro* (Antunes et al. 2012). This antibacterial activity is maintained in the *Galleria mellonella* model. The administration of this drug alone at concentrations mimicking the human therapeutic dose of gallium nitrate used for cancer patients (28 μM) significantly increased the survival of larvae after infection by *A. baumannii* (Antunes et al. 2012). When a vertebrate model, such as murine models of acute and chronic lung infections by *A. baumannii*, was used, gallium nitrate reduced lung injury and bacterial loads in tissues (De Léséleuc et al. 2012).

Combined with colistin and LpxC inhibitors, gallium nitrate showed synergistic activity against *P. aeruginosa* and *K. pneumoniae*, and *A. baumannii*, respectively (Choi et al. 2019; Vinuesa et al. 2021). Most importantly, combining gallium nitrate with minocycline into niosomes dispersed biofilms and treated pneumonia in mice faster than minocycline monotherapy (Shamkani et al. 2023).

Selective Estrogen Receptor Modulator

Regarding selective estrogen receptor modulator (SERM) drugs used in the prevention of osteoporosis and invasive breast cancer in post-menopausal women, tamoxifen has been reported to exhibit activity against *A. baumannii*, *E. coli* and *P. aeruginosa* in both immunocompetent and neutropenic murine models of peritoneal sepsis by decreasing the bacterial loads in the spleen, lungs, and blood, and increasing the mice's survival (Miró Canturri et al. 2021a). Tamoxifen treatment also enhanced the survival of mice injected with a lethal dose of Shiga toxin-producing *E. coli* infections (Selyunin et al. 2019). Of note, tamoxifen metabolites (N-desmethyltamoxifen, 4-hydroxytamoxifen, and endoxifen), produced after tamoxifen metabolism by cytochrome P450 (Hoskins et al. 2009), have presented antibacterial activity *in vitro* with MIC_{90} of 16 mg/L, against a collection of 100 and 47 MDR and pan-drug resistant (PDR) *A. baumannii* and *E. coli* clinical isolates respectively (Miró Canturri et al. 2021b).

In pneumonia and sepsis murine models by MDR *A. baumannii* or *E. coli*, the combination of tamoxifen metabolites with colistin or tigecycline exhibits therapeutic efficacy by decreasing the bacterial loads in the spleen, lungs, and blood and increasing the mice's survival (Herrera Espejo et al. 2024).

Raloxifene, in turn, attenuated *in vitro* and in the *Caenorhabditis elegans* model, the growth and the virulence of *P. aeruginosa* by binding to PhzB2, which is involved in the production of pyocyanin (Ho Sui et al. 2012; Kannon et al. 2024). Furthermore, toremifene has shown efficacy against oral infection caused by *Porphyromonas gingivalis* (Gerits et al. 2017).

Together with colistin, clomiphene showed antimicrobial activity against planktonic *P. aeruginosa* (Torres et al. 2018).

5-fluorouracil

A potent anticancer drug called 5-fluorouracil is indicated for the treatment of different types of solid tumors, and its prodrug, 5-fluorocytosine, has exhibited antibacterial activity against a collection of 5,850 mutants of the PA14 strain of *P. aeruginosa*, revealing positive activity via the regulation of a large number of genes involved in *quorum sensing* and biofilm formation (Ueda et al. 2009; Rangel-Vega et al. 2015; Di Bonaventura et al. 2023). Pharmacological perturbation of thiamine metabolism has been shown to sensitize *P. aeruginosa* to this anticancer drug *in vitro* and in a murine ocular infection model by *P. aeruginosa* (Kim et al. 2022).

In *E. coli*, 5-fluorouracil presented MICs 32-64 mg/L, including against strains carrying $bla_{\text{NDM-5}}$, conferring resistance to carbapenems (Zheng et al. 2024).

Mitomycin C

Mitomycin C, used for several types of carcinomas such as superficial vesical carcinoma (Janeiro-Pais et al. 2009), was effective against *A. baumannii* with an MIC_{50} of 7 mg/L and increased *G. mellonella* survival after infection by *A. baumannii* and *P. aeruginosa* (Cruz-Muñiz et al. 2017; Svedholm et al. 2024). The combination of mitomycin C with tobramycin and ciprofloxacin together and with gentamicin or pentamidine has increased *in vitro* the activity of this anticancer drug against MDR clinical isolates of *A. baumannii*, *P. aeruginosa*, *K. pneumoniae* and *E. coli* (Domalaon et al. 2019; Wu et al. 2020; Svedholm et al. 2024).

Of note, mitomycin C has also been combined with lytic phage vB_KpnM-VAC13, and this combination showed synergistic effects on imipenem-resistant and persister isolates of *K. pneumonaie* (Pacios et al. 2021).

Other Anti-Cancer Drugs

Mitotane, an FDA-approved antineoplastic drug, has been combined with polymyxin B against polymyxin B-resistant *A. baumannii* and *P. aeruginosa*, and has presented synergy with polymyxin B by increasing the activity of polymyxin B *in vitro* and a murine model of burn wound infection by reducing the bacterial load in wounds (Tran et al. 2018a).

In addition, cisplatin, approved for treating several cancers, was found to inhibit *P. aeruginosa* growth and eradicate *E. coli* persister cells through a growth-independent mechanism (Chowdhury et al. 2016; Domalaon et al. 2019).

Gram-Positive Bacteria

Gallium Nitrate

As reported, combination therapies using conventional antibiotics and anticancer drugs are effective methods against resistant bacterial infections. In this context, two reports showed that gallium nitrate enhanced the antibacterial activity of vancomycin by reducing the formation of biofilms, increasing the destruction of biofilms, and the release of reactive oxygen species (ROS) in methicillin-resistant *Staphylococcus aureu*s (MRSA) (Mohammad Hanifeh et al. 2024). Combined with gallium

protoporphyrin, gallium nitrate effectively eliminated mature MRSA biofilms (Xia et al. 2021), increased survival, and decreased MRSA lung burden in mice (Choi et al. 2023).

Selective Estrogen Receptor Modulator

Regarding SERM drugs, some of them have demonstrated remarkable antibacterial activity. For example, tamoxifen metabolites have presented antibacterial activity against methicillin-resistant *S. epidermidis*, *Enterococcus faecalis*, and *E. faecium* clinical isolates by presenting MIC_{90} between 1 and 2 mg/L and bactericidal activity over 24 h (Miró-Canturri et al. 2021c).

Toremifene, in turn, showed antibacterial and antibiofilm activity against *Streptococcus mutans* and *P. gingivalis in vitro* (Gerits et al. 2017). This effect may be explained by incremental increases in membrane permeabilization and consequent membrane damage (Gerits et al. 2017). *In vivo*, toremifene reduced biofilm formation by *S. aureus* in a rat subcutaneous catheter model (De Cremer et al. 2014).

Moreover, clomiphene has demonstrated efficacy against *S. aureus* and *Bacillus subtilis in vitro*, with an MIC value of 8 mg/L, and incubation of *B. subtilis* with this concentration of clomiphene changed its morphology (Farha et al. 2015). Due to its action on the bacterial wall, as mentioned previously, clomiphene exhibits synergy with β-lactams in restoring MRSA susceptibility (Farha et al. 2015).

Unlike tamoxifen, raloxifene has been shown to display an increase in the bactericidal activity of ciprofloxacin in time-kill kinetics of *S. aureus*, prolonged its post-antibiotic effect, and reduced the frequency of spontaneous resistant mutant development (Mahey et al. 2021).

Besides their direct effect on bacteria, SERMs may also modulate the host immune response. It was reported that in an *in vitro* murine systemic model, tamoxifen modified the host response against MRSA infection by stimulating chemotaxis, phagocytosis, and neutrophil extracellular traps (NETs) formation through the modulation of the ceramide pathway (Corriden et al. 2015). Similarly, Flores et al. showed that raloxifene reduced NET formation in human neutrophils and consequently reduced cell death by MRSA (Flores et al. 2016).

Floxuridine and 5-fluorouracil

The FDA-approved anticancer drug floxuridine (mostly used in colorectal cancer) showed direct antibacterial activity by inhibiting the growth of *S. aureus* USA300 at a concentration of 0.0625 mg/L *in vitro* and increasing the survival of mice by 60% in a murine model of blood infection *in vivo* (Yeo et al. 2018). Floxuridine can also have a protective effect on the host by reducing *S. aureus*-mediated killing in human neutrophils (Yeo et al. 2018). Moreover, 5-fluorouracil has exhibited high antibacterial activity against *S. aureus* and *E. faecalis* with a MIC of 8 mg/L (Kikuchi et al. 2024).

Other Anti-Cancer Drugs

Streptozotocin, used for pancreatic islet cell cancer, has been reported to reduce the mortality of mice to 10%, even though it did not affect staphylococcal growth *in*

vitro (Yeo et al. 2018). In combination with floxuridine, streptozotocin has shown a protective effect on the host by reducing *S. aureus*-mediated killing in human neutrophils (Yeo et al. 2018).

Regarding denileukin diftitox, an antineoplasic agent, treatment with this drug slightly reduced the lung bacterial count in mice aerosol-infected with *M. tuberculosis* (Gupta et al. 2017). Adding denileukin diftitox to standard tuberculosis treatments, composed of rifampin, isoniazid, and pyrazinamide, similarly reduced the bacterial burden (Gupta et al. 2017).

In the case of mitomycin C, it was combined with pentamidine, increasing the *in vitro* activity of pentamidine against *E. cloacae* but not against *S. aureus* and *E. faecium* (Wu et al. 2020).

Finally, against *S. aureus* persister cells, Chowdhury et al. showed that cisplatin eradicated these persister cells (Chowdhury et al. 2016).

Mechanism of Action of Anticancer Drugs

Various anticancer agents have shown promise as candidates for repositioning as antimicrobial agents to treat infections caused by Gram-negative and Gram-positive bacteria, both *in vitro* and *in vivo*. A range of drugs with diverse mechanisms of action and targets has been identified, including inhibitors of DNA, RNA, and proteins; quorum-sensing regulators; biofilm formation inhibitors and disruptors; drugs that interact with the cell membrane; drugs that affect iron metabolism; and host immune system modulators. These drugs and their mechanisms of action against critical and high-priority pathogens (*A. baumannii*, *P. aeruginosa*, *Enterobacterales*, and *S. aureus*) are summarized in Table 1 and Figures 1 and 2.

DNA and Protein Inhibition

Regarding DNA and protein inhibitors, mitomycin C binds to DNA during DNA synthesis and inhibits its synthesis and function in *P. aeruginosa* and *E. coli* (Kwan et al. 2015). Cisplatin, in turn, was found to inhibit microbial cell growth (Yuan et al. 2018; Domalaon et al. 2019) through the upregulation of the recA gene in

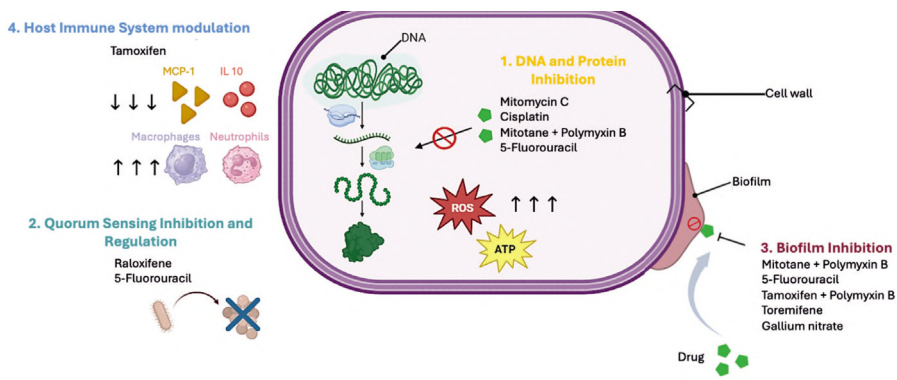

Figure 1. Mechanisms of action of repurposed anticancer drugs in Gram-negative bacteria.

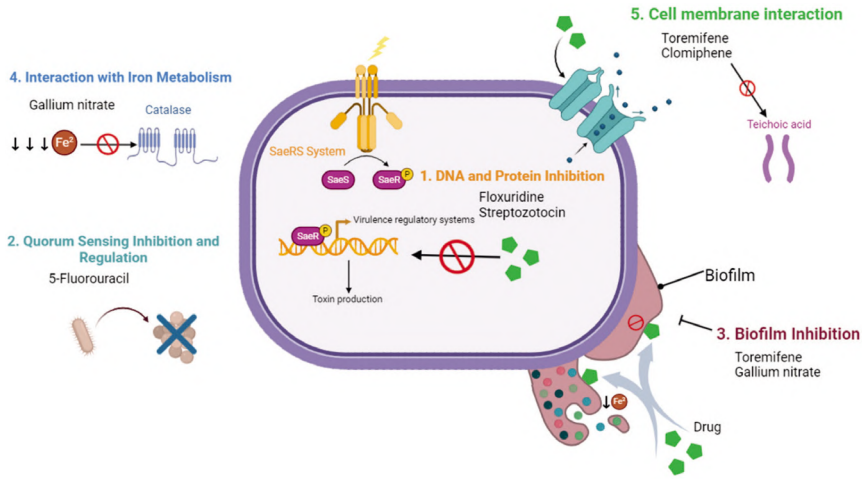

Figure 2. Mechanisms of action of repurposed anticancer drugs in Gram-positive bacteria.

P. aeruginosa, which is important for DNA repair, indicating that cisplatin could interfere with DNA replication (Yuan et al. 2018). Furthermore, the combination of mitotane and polymyxin B significantly perturbed the glycerophospholipid metabolism, the pentose phosphate pathway, the citric acid cycle, and the pyrimidine ribonucleotide biogenesis in polymyxin-resistant *A. baumannii* (Tran et al. 2018b).

In *E. coli*, 5-fluorouracil can act on DNA and protein levels by inducing DNA damage, increasing intracellular ATP levels and the NAD^+/NADH ratio, promoting ROS production, and affecting protein synthesis (Zhang et al. 2024; Kikuchi et al. 2024).

Quorum Sensing Inhibition and Regulation

Quorum sensing inhibition and regulation are reported among the antibacterial properties of anticancer drugs such as 5-fluorouracil and raloxifene. 5-fluorouracil has been proposed as a *quorum-sensing* inhibitor in *P. aeruginosa* and MRSA (Ueda et al. 2009; Imperi et al. 2019; Sedlmayer et al. 2021) by inhibiting *quorum*-sensor autoinducer-2 (Sedlmayer et al. 2021). Additionally, 5-fluorouracil has dual inhibition mechanisms, including functioning as an alternative substrate resulting in miscoding DNA and RNA and inhibiting thymidylate synthase of MDR *A. baumannii* (Cheng et al. 2019). The SERM drug raloxifene binds to PhzB2, which is involved in the production of pyocyanin, a pigment related to both the virulence factor and the *quorum-sensing* signaling molecule in *P. aeruginosa* (Ho Sui et al. 2012).

Biofilm Formation Inhibition

Compared with *quorum sensing*, more drugs act on biofilm formation and interact with the bacterial cell membrane. 5-fluorouracil has been revealed to regulate different genes involved in biofilm formation by *P. aeruginosa* (Ueda et al. 2009). Tran et al. have demonstrated that mitotane in combination with polymyxin B allows

Table 1. Mechanisms of action of repurposed anticancer drugs against Gram-negative and Gram-positive bacteria ⏎

Drug	Clinical Indication	Target Bacteria	Mechanism of Action	Reference
Gallium nitrate	Lymphoma and bladder cancer	*P. aeruginosa*	Effects on iron metabolism	Antunes et al. 2012
		S. aureus	Inhibition of the MRSA catalase and promotion of the extracellular DNA-dependent biofilm dispersion under iron-limited conditions	Choi et al. 2023; Xi et al. 2021
Tamoxifen	Breast cancer	*A. baumannii*, *P. aeruginosa* and *E. coli*	Reduction in the migration of immune cells from bone marrow to blood through the reduction of MCP-1 and IL-18 in the presence of A. baumannii, P. aeruginosa , and *E. coli* Enhancement of the killing activity of macrophages and neutrophils against *A. baumannii* and *E. coli*	Miró Canturri et al. 2021a
		P. aeruginosa	Disruption of the cell envelope biogenesis and inhibition of the aminoarabinose LPS modifications	Hussein et al. 2018
Toremifene	Breast cancer	*S. mutans* and *P. gingivalis*	Membrane permeabilization and damage	Gerits et al. 2017
Raloxifene	Breast cancer	*P. aeruginosa*	Binding to PhzB2, which is involved in the production of pyocyanin, a pigment related to the virulence of *P. aeruginosa*	Ho Sui et al. 2012
Clomiphene	Fertility	*S. aureus*	Inhibition of undecaprenyl diphosphate synthase involved in the synthesis of teichoic acid wall	Farha et al. 2015
5-fluorouracil	Solid tumors	*P. aeruginosa*, *S. aureus*	Inhibition of biofilm formation and *quorum sensing*	Ueda et al. 2009, Imperi et al. 2019
		S. aureus	Inhibition of *quorum sensing* by inhibiting *quorum-sensor* autoinducer-2	Sedlmayer et al. 2021

(*Contd.*)

(*Contd*.)

Drug	Clinical Indication	Target Bacteria	Mechanism of Action	Reference
		E. coli	Induction of DNA damage, increase of intracellular ATP levels and the NAD+/NADH ratio, promotion of ROS production, and affection of the protein synthesis	Zhang et al. 2024, Kikuchi et al. 2024
		A. baumannii	Miscoding DNA and RNA and inhibiting thymidylate synthase	Cheng et al. 2019
Floxuridine	Colorectal cancer	*S. aureus*	Inhibition of the SaeRS two-component system and inhibition of the transcription of other virulence regulatory systems	Yeo et al. 2018
Streptozotocin	Pancreatic islet cell cancer	*S. aureus*	Inhibition of the SaeRS two-component system and inhibition of the transcription of other virulence regulatory systems	Yeo et al. 2018
Mitomycin C	Superficial vesical carcinoma	*E. coli* and *P. aeruginosa*	Binding to DNA during DNA synthesis and causes inhibition of its synthesis and function in *P. aeruginosa* and *E. coli*	Kwan et al. 2015
Cisplatin	Cancer	*P. aeruginosa*	Upregulation of the *recA* gene of *P. aeruginosa*, which is known to be important for DNA repair	Yuan et al. 2018
		A. baumannii	Perturbation of glycerophospholipid metabolism, pentose phosphate pathway, citric acid cycle, and pyrimidine ribonucleotide biogenesis after incubation with polymyxin B	Tran et al. 2018b
Mitotane	Cancer	*A. baumannii*, *P. aeruginosa* and *K. pneumoniae*	Permeabilization of the outer membrane of *A. baumannii*, *P. aeruginosa*, and *K. pneumoniae*	Tran et al. 2018

mitotane to enter inside *A. baumannii*, *P. aeruginosa*, and *K. pneumoniae* through the permeabilization of the outer membrane by polymyxin B (Tran et al. 2018a). In the same line, tamoxifen, in combination with polymyxin, disrupts the cell envelope biogenesis and inhibits the aminoarabinose LPS modifications of polymyxin-resistant *P. aeruginosa* (Hussein et al. 2018). Finally, toremifene showed antibacterial and antibiofilm activity against *S. mutans* and *P. gingivalis in vitro* (Gerits et al. 2017). This effect may be explained by incremental increases in membrane permeabilization and consequent membrane damage (Gerits et al. 2017).

Regarding the interaction with iron metabolism, Antunes et al. have demonstrated that virulent bacteria can acquire iron in the blood and tissues (Antunes et al. 2012). Given the essential role of iron in bacterial physiology and pathogenicity, iron uptake and metabolism have become attractive targets for developing new antibacterial agents (Ballouche et al. 2009; Foley and Simeonov 2012). The ion gallium [Ga(III)], a ferric iron [Fe(III)] mimetic, has been shown to inhibit the growth of many bacterial species by interfering with iron-dependent metabolic pathways. Combining gallium nitrate with gallium protoporphyrin against MRSA under iron-limited conditions inhibited the MRSA catalase and promoted extracellular DNA-dependent biofilm dispersion (Xi et al. 2021; Choi et al. 2023). Therefore, gallium drugs *in vitro* and *in vivo* have gained special interest in the fight against MDR bacterial infections (De Léséleuc et al. 2012; Antunes et al. 2012; Runci et al. 2017; Hijazi et al. 2018; Rezzoagli et al. 2018; Choi et al. 2023).

Host Immune System Modulation

Some anticancer drugs that modulate the host immune system have reported antibacterial activity against Gram-negative bacteria. Tamoxifen can reduce the migration of immune cells from bone marrow to blood through the reduction of monocyte chemoattractant protein 1 (MCP-1) and IL-18 in a murine model of sepsis by *A. baumannii*, *P. aeruginosa*, and *E. coli* (Miró Canturri et al. 2021a). Moreover, tamoxifen has been shown to enhance the killing activities of macrophages and neutrophils against *A. baumannii* and *E. coli in vitro* (Miró Canturri et al. 2021a).

Clinical Application of Anticancer Drugs Against Infectious Agents

Even though repurposed anticancer drugs showed promising preclinical data for the treatment of bacterial infections, at present, clinical trials on the repositioning of anticancer drugs mainly concern the treatment of Alzheimer's disease (Ancidoni et al. 2021). Only four clinical studies have been performed or are currently underway to repurpose gallium nitrate and citrate as antibacterial agents (Table 2).

A Phase I clinical trial was started in 2010 to test the pharmacokinetics, safety, and tolerability of intravenous infusion of gallium nitrate in patients with chronic cystic fibrosis caused by *P. aeruginosa* (Clinical trial_NCT01093521 2015). The results of this clinical trial indicated the safety and efficacy of gallium nitrate in cystic fibrosis patients with chronic *P. aeruginosa* lung infections (Goss et al. 2018). At the same clinical stage, a new study has been launched to assess the safety and

Table 2. List of repurposing anticancer drugs under clinical trial against bacterial infections ⏎

Drug	Clinical Indication	Target Bacteria	New Clinical Indication	Clinical Trial Phase	Clinical Trial Identifier
Gallium nitrate	Cancer-associated hypercalcemia	*P. aeruginosa*	Cystic fibrosis	Phase I	NCT01093521
Gallium nitrate	Cancer-associated hypercalcemia	*P. aeruginosa*	Cystic fibrosis	Phase I	NCT04294043
Gallium nitrate	Cancer-associated hypercalcemia	*P. aeruginosa*	Cystic fibrosis	Phase II	NCT02354859
Gallium citrate	Hodgkin's disease, lymphoma, or lung cancer diagnostic agent	*P. aeruginosa*	Cystic fibrosis	Phase II	NCT03669614

tolerability of two 5-day infusion cycles of gallium nitrate in adult patients with cystic fibrosis who are infected with nontuberculous mycobacteria (Clinical trial_NCT04294043 2021)

A clinical trial Phase II was started in 2016, evaluating the capacity of gallium nitrate to improve pulmonary function in 60 patients with cystic fibrosis by *P. aeruginosa*. The results of this trial showed that treatment with gallium nitrate increased the forced expiratory volume in these patients (Clinical trial_NCT02354859 2016). At the same clinical stage, an inhalable form of gallium citrate, AR-501, which functions as an iron analog, effectively starving bacteria by depriving them of the essential iron required for their growth and survival, has been given to 41 cystic fibrosis patients who have well tolerated all repeated weekly doses; and all dose levels were tested (Clinical trial_NCT03669614).

Conclusions and Perspectives

In recent years, significant advancements have been made in repurposing anticancer drugs for treating bacterial infections. Many compounds have shown promising preclinical results; however, this development has remained mainly at the preclinical stage. Further clinical studies are crucial to address the urgent need for new bacterial infection treatments.

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CHAPTER

6

Repurposing Drugs for Effective Treatment of Tuberculosis: A Promising Approach

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Introduction

Tuberculosis (TB) is a severe infectious disease primarily affecting the lungs, though it can spread to other body parts, caused by the bacterium *Mycobacterium tuberculosis* (*M. tb*). Despite being both preventable and treatable, TB remains one of the top ten leading causes of death globally. It is the deadliest disease caused by a single infectious agent, surpassing the mortality rates of both human immunodeficiency virus (HIV) and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Shariq et al. 2022; WHO 2023). The World Health Organization (WHO) reports that around 10 million people contract TB annually, with approximately 1.5 million deaths each year (WHO 2023). TB is a significant global health issue owing to its widespread transmission, its impact on vulnerable populations as a chronic or co-morbid disease, and the challenges it poses to public health systems (Malik et al. 2023). TB is predominantly a concern in nations with low and middle incomes, where it is frequently associated with issues such as poverty, poor nutrition, and substandard healthcare systems (Lee et al. 2022; Wu et al. 2023). To effectively combat TB, novel

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strategies must be adopted. Drug repurposing is a promising approach that can be leveraged to develop innovative treatments for TB.

Drug repurposing offers a promising strategy in the fight against *M. tb*, the causative agent of TB. This approach involves identifying new therapeutic uses for existing drugs, significantly reducing the time and cost of drug development (Fatima et al. 2021). Given the rise of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains of *M. tb*, finding effective treatment options is urgent (Seung et al. 2015). Traditional drug discovery processes are slow and expensive, often taking over a decade to develop new treatments. In contrast, repurposed drugs have already passed safety and toxicity assessments, enabling quicker deployment. Recent studies highlight several repurposed drugs, such as metformin, statins, and anti-psychotic agents, which enhance host immune responses or target bacterial pathways. These drugs can either be used in combination with standard TB therapies or offer alternative mechanisms to combat drug resistance. Their integration into TB treatment protocols could be a game-changer in reducing the global burden of TB (Hua et al. 2022; Kulkarni et al. 2023).

Challenges and Strategies in Combating TB: A Focus on Drug Resistance and Co-Infections

TB is more common among individuals with compromised immune systems, such as those with HIV infections, and in people with metabolic conditions, like diabetes. TB poses a significant threat in areas where HIV co-infection rates are high, as individuals with weakened immune defenses are more likely to develop active TB (Figure 1) (Getahun et al. 2020; Morales et al. 2022). The disease is highly contagious and spreads through the air when an infected person coughs, sneezes, or speaks (Figure 1). This ease of transmission, coupled with its long incubation period, makes TB control challenging. Furthermore, the stigma associated with TB can deter individuals from seeking treatment, contributing to the persistence and spread of the disease (Figure 1) (WHO 2023; Nguyen et al. 2024).

A significant obstacle in the fight against TB is the extended and intricate treatment protocol. For TB that responds to standard medications, the prescribed therapy consists of multiple antibiotics administered over a minimum of six months. This prolonged treatment duration often results in patients struggling to adhere to the regimen, which increases the likelihood of unsuccessful treatment outcomes and the emergence of drug-resistant TB strains (Seung et al. 2015; Zarin et al. 2024). The increasing prevalence of TB resistance to drugs is a worldwide issue of growing concern. When TB bacteria develop resistance to isoniazid and rifampicin, the two most potent first-line anti-TB medications, MDR-TB is produced. Treatment for MDR-TB necessitates the use of second-line drugs, which are generally less efficacious, more harmful, and require an extended treatment period, usually lasting between 18 and 24 months. The WHO's recent recommendations for treating MDR-TB are given in Table 1 (Prasad et al. 2018). XDR-TB represents a more advanced and severe form of infection. In this stage, the bacteria have developed resistance to both primary treatment options and most secondary medications, severely limiting the available therapeutic approaches (Seung et al. 2015). The rise in drug-resistant

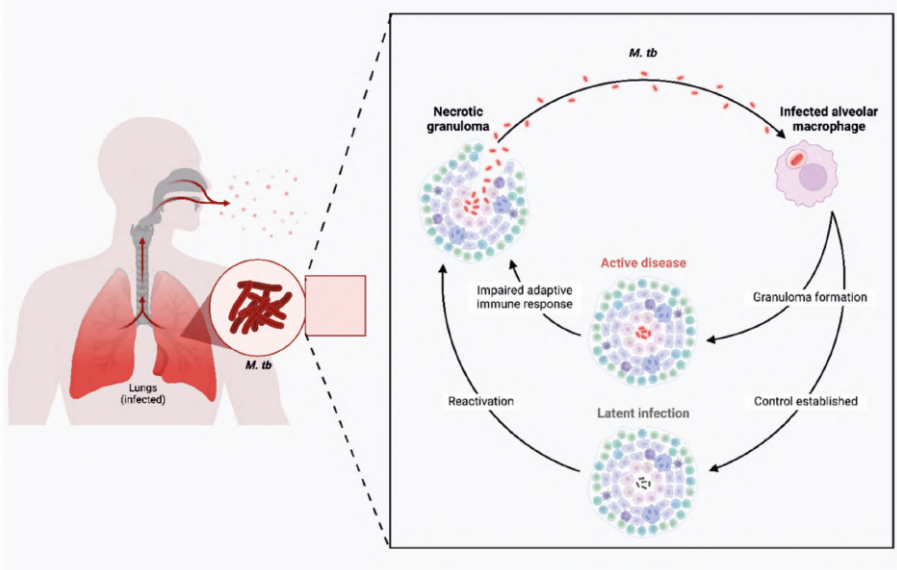

Figure 1. Schematic representation of *M. tb* infection in humans. *M. tb* transmission occurs mainly through airborne particles expelled when an infected individual coughs or sneezes. Upon inhalation, the bacteria travel to the lung alveoli, where macrophages consume them. However, *M. tb* has developed strategies to persist within these immune cells, resulting in latent infection. The progression to active TB occurs when the bacteria reproduce and induce symptoms like coughing, elevated body temperature, and loss of body mass. This phase is marked by the emergence of tubercles, also known as granulomas, which are aggregations of immune cells attempting to contain the bacterial spread. If left untreated, active TB can spread via the bloodstream to other bodily organs. Latent tuberculosis infection (LTBI) occurs when the immune system successfully controls the infection but does not completely eliminate it. In LTBI, *M. tb* remains dormant within granulomas; the person has no symptoms and cannot spread the bacteria to others. However, LTBI can reactivate, particularly if the immune system becomes compromised. Complete control of TB involves a combination of early detection, effective treatment, and preventive measures. Treatment typically includes a multi-drug regimen lasting several months. Vaccination with the BCG vaccine can also help reduce the risk of TB, especially in high-risk populations. ⏎

TB is driven by several factors, including inadequate or incomplete treatment, poor-quality drugs, and transmission of resistant strains. In many parts of the world, healthcare systems are ill-equipped to manage MDR-TB and XDR-TB, resulting in poor outcomes and continued transmission of resistant strains (Lange et al. 2014).

Worldwide initiatives to tackle TB, such as the WHO End TB strategy, aim to decrease TB occurrence and death rates, as well as address the challenge of drug-resistant TB. These programs prioritize quick identification, immediate treatment, and tackling the societal factors that influence health and contribute to TB transmission (John 2019). In the battle against TB, essential elements include innovative diagnostic methods, reduced treatment durations, and the creation of more potent vaccines. Despite advancements in combating TB, it remains a significant worldwide health issue. The emergence of drug-resistant strains further hinders efforts to manage the

Table 1. WHO's recent recommendations for the treatment of drug-resistant TB ⏎

Drug	Recommendation
Bedaquiline	• Should be included in longer MDR-TB regimens for patients aged 18 years or more • May be included for patients aged 6–17 years • May be used in all-oral regimens for children below 6 years
Linezolid	• Should be included in the treatment of MDR/RR-TB patients on longer regimens
Clofazimine	• May be included in the treatment of MDR/RR-TB patients on longer regimens
Cycloserine or Terizidone	• May be included in the treatment of MDR/RR-TB patients on longer regimens
Delamanid	• May be included for MDR/RR-TB patients aged 3 years or more on longer regimens • May be used in children below 3 years as part of longer regimens
Levofloxacin or Moxifloxacin	• Should be included in the treatment of MDR/RR-TB patients on longer regimens
Imipenem–cilastatin or Meropenem	• May be included in the treatment of MDR/RR-TB patients on longer regimens
Amikacin	• May be included for patients aged 18 years or more when susceptibility is demonstrated and monitoring is possible
Ethionamide or Prothionamide	• May be included, only if bedaquiline, linezolid, clofazimine, or delamanid are not used or better options are not possible
P-aminosalicylic acid	• May be included only if bedaquiline, linezolid, clofazimine, or delamanid are not used or better options are not possible

MDR-TB: Multidrug-resistant tuberculosis; RR-TB: Rifampicin-resistant tuberculosis

disease, emphasizing the necessity for ongoing investment in scientific research, medical infrastructure, and public health initiatives. Tackling TB demands a unified global approach that addresses the clinical and societal factors contributing to the disease's persistence (Scriba et al. 2020; Alsayed and Gunosewoyo 2023).

Prolonged TB Treatment: Barriers to Adherence and the Rising Threat of MDR-TB and XDR-TB

The primary medications used include isoniazid, rifampicin, pyrazinamide, and ethambutol, which are effective against most TB strains. A major obstacle in current TB treatment is the toxicity of the drugs used. TB medications can cause serious adverse effects, including damage to the liver, problems with the digestive system, and neurological complications. Isoniazid and rifampicin, for instance, are associated with hepatotoxicity, which can be fatal if not carefully monitored. These adverse reactions frequently result in patients not adhering to their treatment regimens,

particularly those already managing other health conditions (Zhao et al. 2020). The extended treatment duration presents another major challenge. The requirement for patients to consume multiple medications daily for at least six months imposes a considerable burden. Many individuals, especially those in resource-limited environments, struggle to finish the entire course of treatment, resulting in incomplete therapy and potential drug resistance.

Furthermore, the prolonged treatment period places a strain on healthcare systems, necessitating ongoing supervision and assistance to ensure patients adhere to their prescribed medication regimen (Limenh et al. 2024). MDR-TB shows resistance to isoniazid and rifampicin, the two most effective first-line medications. XDR-TB exhibits resistance not only to isoniazid and rifampicin but also to fluoroquinolone and at least one second-line injectable drug, such as amikacin, kanamycin, or capreomycin. Treating MDR-TB and XDR-TB necessitates the use of second-line drugs, which are less efficacious and more harmful, requiring extended treatment periods (sometimes up to two years). These medications are also costlier, making treatment unattainable for many patients in low- and middle-income nations (Table 1) (Vishwakarma et al. 2023; Vanino et al. 2023).

Unlocking New Potentials: The Power of Drug Repurposing in the Battle Against *M. tb*

The drug repurposing capitalizes on the established understanding of already used medications' safety profiles, pharmacological properties, and adverse effects, making it a viable approach for pharmaceutical development (Figure 2). Repurposing drugs has emerged as a crucial tactic in the quest for novel TB treatments, particularly in light of the obstacles associated with TB therapy, such as antimicrobial resistance and extended treatment period (Singh et al. 2023a). The creation of novel TB medications has been hindered by the substantial expenses and lengthy timelines associated with conventional drug discovery methods. As a result, repurposing existing drugs has emerged as a promising approach. Since approved drugs have already undergone extensive safety and efficacy evaluations for other conditions, scientists can skip certain initial phases of drug development. This significantly expedites the process of introducing new tuberculosis treatments to patients (Sharma et al. 2023). For instance, several non-TB drugs, such as linezolid, gatifloxacin, levofloxacin, moxifloxacin, meropenem, and clofazimine, originally developed for other diseases, have shown effectiveness in treating drug-resistant TB and are now included in TB treatment regimens (Padmapriyadarsini et al. 2022; Sharma et al. 2023).

One of the most significant advantages of drug repurposing is cost reduction. Traditional drug development is costly, often requiring over a billion dollars in investment before a new drug can reach the market. The extensive research, development, and clinical testing necessary to verify a drug's safety and effectiveness accounts for this (Low et al. 2020). Drug repurposing, however, reduces these costs by utilizing drugs that have already passed many of these stages. As the safety profile of the drug is already known, resources can be focused on testing the efficacy of the drug against TB, leading to substantial cost reductions. Drug repurposing also significantly shortens the timeline for developing new medicaments (Krishnamurthy

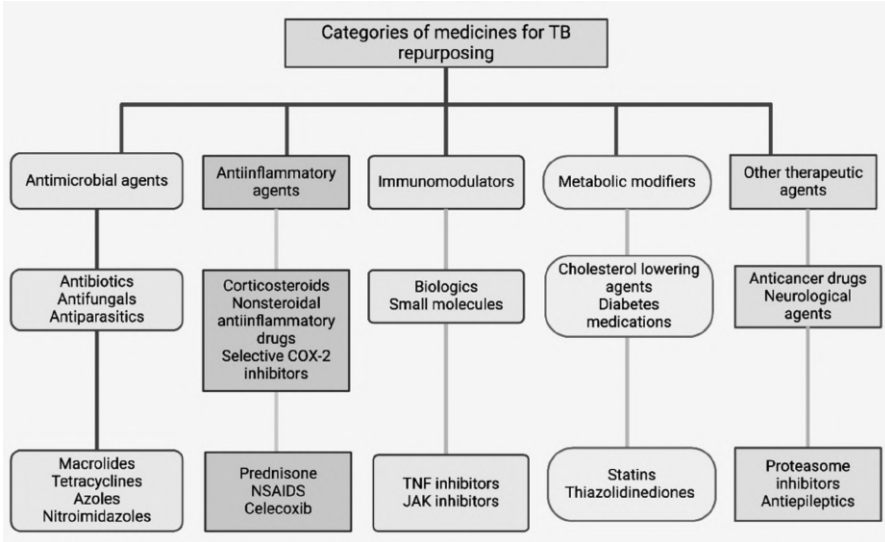

Figure 2. The flow diagram illustrates the potential categories of medicines that may be repurposed for treating *M. tb*. The central node, "Categories of Medicines for TB Repurposing," branches into five main categories: (1) Antimicrobial substances encompass various subgroups, such as antibiotics (for example, macrolides and tetracyclines), antifungal medications (like azoles), and drugs that combat parasites (including nitroimidazoles). (2) Another category is anti-inflammatory medications, which include both corticosteroids (such as prednisone) and non-steroidal anti-inflammatory drugs (NSAIDs). The latter group also contains selective COX-2 inhibitors, with celecoxib being an example. (3) Immunomodulators: divided into biologics (e.g., TNF-α inhibitors such as infliximab) and small molecules (e.g., JAK inhibitors such as tofacitinib). (4) Metabolic modifiers: encompasses cholesterol-lowering agents (e.g., statins) and diabetes medications (e.g., thiazolidinediones). (5) Other therapeutic agents: features anti-cancer drugs (e.g., proteasome inhibitors such as bortezomib) and neurological agents (e.g., antiepileptics such as valproic acid). ⏎

et al. 2022; Kulkarni et al. 2023). Traditional drug development can take over a decade, from the discovery phase to approval.

In contrast, repurposed drugs can often reach the market within a fraction of the time required to develop a new chemical entity. This plays a crucial role in combating TB, where new medications are desperately needed due to the escalating prevalence of drug-resistant TB strains (Krishnamurthy et al. 2022). Researchers can swiftly identify and deploy new therapeutic strategies by utilizing existing medications in novel ways, potentially saving lives. Furthermore, repurposed drugs offer the benefit of having well-established safety profiles and pharmacokinetic characteristics. This knowledge reduces the risk of unexpected adverse effects, making the clinical trial process more straightforward and potentially reducing the time required for regulatory approval (Cha et al. 2018). By repurposing drugs from different therapeutic areas, researchers can explore new mechanisms of action that might be effective against *M. tb*, offering new hope for patients who have exhausted other treatment options (Fatima et al. 2021).

Redefining the Fight: The Urgent Need for Novel Therapeutic Approaches Against *M. tb*

The pressing demand for novel therapeutic approaches to enhance TB treatment outcomes cannot be overstated. A top priority is the creation of innovative medications that are more potent, less harmful, and capable of reducing treatment duration. Another potential strategy is the development of host-directed therapies (HDTs), which aim to enhance the body's immune response to TB rather than directly targeting bacteria. This strategy may help limit the emergence of drug resistance and enhance the effectiveness of treatments (Kaufmann et al. 2018; Cubillos-Angulo et al. 2022). HDT offers a novel strategy for TB by boosting the body's immune defenses against *M. tb*. In contrast to conventional antibiotics that directly attack bacteria, HDT works by adjusting the host's immune system to facilitate infection clearance. Recent studies have highlighted HDT's crucial role in managing TB effectively. For instance, the use of immunomodulatory agents, such as interferon-gamma (IFN-γ) and granulocyte-macrophage colony-stimulating factor (GM-CSF), has shown promise in enhancing the effectiveness of conventional therapies and reducing the duration of treatment (Young et al. 2020; Cubillos-Angulo et al. 2022; Zhao et al. 2023). These substances can stimulate macrophage activity, boost the synthesis of peptides with antimicrobial properties, and strengthen the host's immune system's capacity to regulate bacterial growth.

Moreover, emerging research has highlighted the potential of targeting host pathways involved in immune regulation, such as those related to autophagy and inflammasome activation (Shariq et al. 2021). For example, recent studies have suggested that manipulating the autophagy pathway can improve the ability of macrophages to eliminate *M. tb* (Thakur et al. 2019; Watson et al. 2020; Zhang et al. 2021; Chen et al. 2023). Overall, HDT offers a promising adjunct to conventional TB therapies, potentially improving treatment outcomes and addressing drug-resistance challenges. Continued research and clinical trials are essential to validate these approaches and integrate them into standard TB treatment regimens (Liebenberg et al. 2022; Cubillos-Angulo et al. 2022). Developing new, more effective vaccines to replace the current Bacillus Calmette-Guérin (BCG) vaccine is essential for preventing and controlling TB. Although existing TB treatments have shown success in many instances, they face significant challenges, highlighting the need to investigate alternative therapeutic methods (Scriba et al. 2020). Drug repurposing and the development of host-directed therapies represent promising avenues for improving TB treatment outcomes and addressing the menace of drug-resistant TB (Kolloli and Subbian 2017; Tiberi et al. 2018a).

Strategic Drug Repurposing for TB Control

Drug repurposing for TB relies heavily on *in silico* and *in vitro* screening methods. Computational techniques, known as *in silico* screening, are employed to forecast drug-target interactions and identify potential candidates for repurposing (Singh et al. 2021). Researchers can predict which drugs might inhibit crucial *M. tb* proteins

by examining the structural characteristics of drugs and their known targets (Agamah et al. 2020; Blundell et al. 2020; Halder and Elma 2021). Molecular docking simulations have been widely used to screen existing drugs against *M. tb* enzymes, such as those involved in cell wall synthesis (Rastogi et al. 2023). This approach reduces the number of candidates that need to be tested in the laboratory, thereby making the drug discovery process more efficient (Campanico et al. 2018; Ejalonibu et al. 2021; Verma et al. 2023). *In vitro* screening involves testing existing drugs against *M. tb* in laboratory settings, focusing on their ability to inhibit bacterial growth. This approach allows researchers to rapidly identify potential anti-TB compounds among various drugs (Nazim et al. 2023). For instance, high-throughput screening (HTS) has been employed to test large libraries of drugs, leading to the identification of several candidates with anti-TB activity (Bhat et al. 2018; Ejalonibu et al. 2021; Hanscheid et al. 2024). Clinical observation is another crucial strategy in drug repurposing. This approach involves identifying drugs that exhibit anti-TB effects during use in other diseases. Sometimes, patients receiving treatment for unrelated conditions may show unexpected improvements in TB symptoms, leading to recognition of the potential of the drug as an anti-TB agent. This serendipitous discovery process can reveal new avenues for TB treatment, notably when the underlying mechanism of the drug's effect on *M. tb* is lacking (Battah et al. 2019). For example, certain nonsteroidal anti-inflammatory drugs (NSAIDs) have been found to have anti-TB effects in patients treated for inflammatory conditions. Clinical observations allow researchers to tap into real-world data and identify potential candidates that may not have been discovered through traditional screening methods (Maitra et al. 2016; Kroesen et al. 2017).

Reviving the Arsenal: Drug Repurposing for Combatting *M. tb*

Several drugs have been successfully repurposed for TB treatment, and they fall into different categories, such as antibiotics, non-antibiotic drugs, and host-directed therapies. Originally developed to combat infections caused by gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA), the oxazolidinone antibiotic linezolid has found new applications in treating TB, particularly in cases of MDR and XDR-TB. This antibiotic functions by attaching to the bacterial ribosome, thereby impeding protein synthesis. When used with other anti-TB medications, linezolid has shown effectiveness in eliminating *M. tb* infections (Figure 3) (Stephanie et al. 2021). Clofazimine is an antibiotic which is generally used to treat leprosy. Its anti-TB activity was discovered through *in vitro* screening, in which it was found to inhibit *M. tb* by disrupting the bacterial cell membrane. Clofazimine is particularly effective against MDR-TB and is included in several treatment regimens for drug-resistant TB strains. Its unique mechanism of action and ability to penetrate TB lesions make it a valuable addition to the anti-TB arsenal (Lechartier and Cole 2015; Stadler et al. 2023).

The antidiabetic medication metformin has garnered interest due to its potential applications in TB treatment. While not directly killing bacteria, metformin has

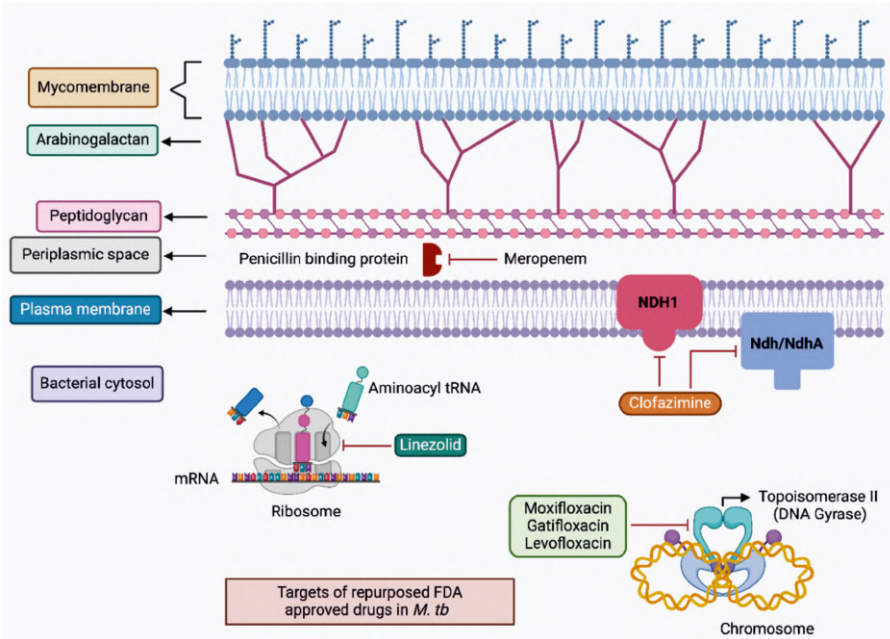

Figure 3. Linezolid, levofloxacin, gatifloxacin, moxifloxacin, meropenem, and clofazimine are antibiotics with distinct mechanisms of action against *M. tb*. Bacterial protein synthesis is impeded by linezolid through its interaction with the 50S ribosomal subunit's 23S rRNA, which prevents the formation of the functional 70S initiation complex. The fluoroquinolones levofloxacin, gatifloxacin, and moxifloxacin disrupt bacterial DNA synthesis by inhibiting topoisomerase II (DNA gyrase) in *M. tb*, an enzyme crucial for DNA replication and repair. Meropenem, a broad-spectrum carbapenem antibiotic, hinders bacterial cell wall synthesis by attaching to penicillin-binding proteins (PBPs), disrupting the peptidoglycan layer and causing cell lysis and death. Clofazimine, functioning as a phenazine dye, employs multiple mechanisms, including DNA binding, bacterial cell membrane disruption, and induction of oxidative stress in *M. tb*. It also exhibits immunomodulatory effects and inhibits electron transport through the inner membrane, reducing proton motive force. These medications can be utilized in various combinations to treat MDR-TB and XDR-TB based on the susceptibility profile of the specific strain. ⏎

demonstrated an ability to boost the body's immune response against *M. tb* by altering mitochondrial function and decreasing inflammation. Research indicates that metformin might enhance TB treatment outcomes by stimulating autophagy, a cellular mechanism that aids in eliminating bacteria within cells. Consequently, metformin shows promise as a supplementary therapy for TB (Marupuru et al. 2017; Bohme et al. 2020; Rodriguez-Carlos et al. 2020). Verapamil, a calcium channel blocker used to treat hypertension, has been repurposed for TB owing to its ability to enhance the efficacy of other anti-TB drugs. It does so by inhibiting the *M. tb* efflux pumps, responsible for expelling drugs from bacterial cells and contributing to drug resistance. By blocking these pumps, verapamil increases the intracellular concentration of antibiotics, making them more effective against *M. tb*. This strategy

of combining verapamil with existing anti-TB drugs is particularly promising for tackling drug-resistant TB (Chen et al. 2018; Meregildo-Rodriguez et al. 2024).

Certain medications, such as statins, have been repurposed to treat TB due to their immune-modulating effects, although they are traditionally prescribed for cholesterol reduction. Statins help reduce inflammatory responses and improve the host's capacity to manage *M. tb* infection by facilitating the elimination of infected macrophages (Ahmed et al. 2020; Matteucci et al. 2022). Drugs that enhance autophagy, such as rapamycin, have shown promise as host-directed therapies for TB. Autophagy is a cellular process that helps degrade intracellular pathogens, and enhancing this process can improve the host's ability to eliminate *M. tb* (Paik et al. 2019; Shariq et al. 2023b). Rapamycin, developed initially as an immunosuppressant, induces autophagy and enhances the clearance of *M. tb* in experimental models (Singh and Subbian 2018).

Decoding Antibacterial Mechanisms: Repurposed Drugs Against *M. tb*

The mechanisms by which repurposed drugs act against TB can be broadly categorized according to their antibacterial mechanisms, where drugs target *M. tb* directly, and host-targeted mechanisms, where drugs enhance the host immune response to reduce *M. tb* survival (Kumar et al. 2019). Comprehending these processes is essential for maximizing the effectiveness of repurposed medications in treating TB. Drugs repurposed for their antibacterial effects against *M. tb* typically target vital bacterial functions, including the synthesis of cell walls, production of proteins, and metabolic routes (Figure 2) (Mazlan et al. 2021; Ayodele et al. 2023).

The cell wall of *M. tb* is a critical structure that provides protection and contributes to the bacterium's resistance to environmental stress and antibiotics. Drugs that inhibit cell wall synthesis are particularly effective in weakening *M. tb's* defense. Linezolid primarily targets protein synthesis and indirectly disrupts cell wall synthesis by limiting the production of essential proteins required for cell wall maintenance. This dual action makes linezolid effective against drug-resistant TB strains (Gan et al. 2023). Clofazimine has been repurposed for TB because of its unique mechanism of action against *M. tb*. Clofazimine employs two mechanisms to combat bacteria. First, it induces the production of reactive oxygen species (ROS), which cause oxidative stress and damage the bacterial cell membrane. Second, it inserts itself into bacterial DNA, inhibiting both replication and transcription processes. The combined effect of these actions weakens the cell wall structure, ultimately resulting in the death of the bacteria (Stadler et al. 2023).

M. tb relies on various metabolic pathways to survive, especially during the latent phase of infection when the bacterium is non-replicating. Drugs that disrupt these pathways can effectively reduce *M. tb* survival. Although bedaquiline was initially developed as an anti-TB drug, its mechanism of action illustrates its potential to target bacterial metabolism. Bedaquiline inhibits *M. tb's* ATP synthase by targeting its c-subunit, which differs structurally from the human enzyme. This specificity disrupts the bacterial energy production, starving it of ATP. The ATP synthase in human mitochondria, while similar, has a different protein composition,

reducing bedaquiline's affinity and minimizing effects on host cells. Although off-target effects on mammalian cells can occur, they are generally less severe compared to their potent action on *M. tb*. This mechanism is particularly effective against drug-resistant and dormant *M. tb*, making bedaquiline a valuable addition to TB treatment regimens (Field 2015; Egorova et al. 2021; Verma et al. 2022). Metformin influences the metabolism of the host and indirectly impacts *M. tb*. Metformin alters host metabolism by stimulating AMP-activated protein kinase (AMPK), a crucial regulator of energy balance in cells. This alteration diminishes the availability of nutrients essential for *M. tb's* survival. Furthermore, metformin boosts the host's immune defenses, thereby hindering *M. tb's* ability to flourish (Goel et al. 2022).

Fluoroquinolones such as levofloxacin, gatifloxacin, and moxifloxacin have been suggested as potential treatments for MDR-TB due to their wide-ranging antibacterial properties and efficacy against *M. tb*. These medications function by inhibiting two crucial bacterial enzymes: DNA gyrase (gyrA) and topoisomerase IV (parC). These enzymes play vital roles in DNA replication, transcription, and repair processes, making them essential targets for antibacterial action (He and Li 2022; Naidoo et al. 2017). In *M. tb*, DNA gyrase is the primary target because it lacks topoisomerase IV. Fluoroquinolones bind to the gyrase-DNA complex, stabilizing it and preventing the relaxation of supercoiled DNA, which is necessary for replication and transcription. This ultimately leads to the generation of double-stranded DNA breaks, inhibition of bacterial proliferation, and induction of cell death (Figure 3) (Hooper and Jacoby 2016). Human cells have structurally different enzymes, making fluoroquinolones less effective against host gyrases, ensuring selective bacterial inhibition with minimal host toxicity (Hooper and Jacoby 2016). Levofloxacin, a third-generation fluoroquinolone, is a second-line treatment for MDR-TB. It is highly effective against *M. tb*, with favorable pharmacokinetics that allows good tissue penetration, including the lungs, where TB bacteria predominantly reside (Sarathy et al. 2019). Gatifloxacin, another third-generation fluoroquinolone, has demonstrated potent activity against *M. tb* with a similar mechanism of action. Although it has been widely explored for MDR-TB therapy, concerns about adverse effects, including dysglycemia, have limited its broader use. However, this remains an option for specific drug-resistant TB regimens (He and Li 2022). Moxifloxacin, a fourth-generation fluoroquinolone, is considered superior to other fluoroquinolones because of its enhanced potency against *M. tb*. Moxifloxacin demonstrates enhanced bactericidal properties and effectiveness at reduced dosages, positioning it as a crucial option for treating MDR-TB and XDR-TB. Researchers have conducted various clinical trials investigating moxifloxacin's potential to reduce treatment duration and boost regimen effectiveness (Johnson et al. 2024). Adapting these fluoroquinolones has offered a crucial alternative for treating drug-resistant TB, though vigilant monitoring of side effects and potential resistance development is essential.

The carbapenem antibiotic meropenem, known for its wide-ranging effectiveness, has garnered increased attention for its potential use against *M. tb*, especially in cases of drug-resistant TB. This antibiotic functions by disrupting the formation of bacterial cell walls. Specifically, meropenem acts on penicillin-binding proteins (PBPs), which play a vital role in the cross-linking processes essential for maintaining the structural stability of bacterial cell walls (Terreni et al. 2021). In

M. tb, meropenem specifically inhibits PBP3, leading to defective cell wall synthesis, which ultimately compromises bacterial cell viability and causes cell death (Figure 3). The use of meropenem in TB treatment is particularly promising because of its ability to act on slowly replicating and non-replicating bacteria, which are common in chronic TB infections. It is typically used with an adjuvant, such as clavulanate, to inhibit beta-lactamase enzymes that degrade carbapenems, enhancing its effectiveness against resistant TB strains. This combination has shown potential in clinical settings, offering a valuable option for the multidrug-resistant TB treatment arsenal (Kumar et al. 2012; England et al. 2012).

Targeting the Host: Repurposed Drugs and Their Mechanisms in TB Control

Host-targeted therapies aim to boost the body's natural defenses against *M. tb*, reduce the bacterial burden, and improve treatment outcomes. Metformin increases the body's capacity to combat *M. tb* infection by stimulating autophagy, a cellular mechanism that breaks down and reuses internal components, including pathogens. Metformin facilitates the elimination of *M. tb* from infected cells by activating autophagy in the host's immune cells. Additionally, metformin diminished the persistent inflammation linked to the progression of TB. This dual action of modulating metabolism and enhancing immune response makes metformin a promising adjunctive therapy for TB (Yew et al. 2020; Shariq et al. 2023a; Malik et al. 2024a; Malik et al. 2024b). Statins inhibit the mevalonate pathway, which is involved in cholesterol synthesis and inflammation. Statins help prevent excessive tissue damage that can occur during TB infection by reducing inflammation (Dutta et al. 2020; Morofuji et al. 2022). Additionally, statins enhance the ability of macrophages to kill *M. tb* by promoting phagolysosome formation, a process that leads to the degradation of engulfed bacteria. This ability to modulate immune responses makes statins a potential adjunct therapy for TB, particularly in cases of chronic infection (Guerra-De-Blas et al. 2018).

Strengthening Host Immunity: Enhancing Cellular Defenses with Repurposed Drugs Against *M. tb*

Some repurposed drugs enhance the host's cellular defense mechanisms, making it more difficult for *M. tb* to establish and maintain infection (Selvapandiyan et al. 2023). Rapamycin inhibits the mammalian target of the rapamycin (mTOR) pathway, inducing autophagy and leading to the degradation of intracellular pathogens, including *M. tb*. This enhanced autophagic activity helps the host to control TB infection, particularly in cases where traditional antibiotics may be less effective. Additionally, rapamycin has been shown to reduce the formation of granulomas, immune structures that *M. tb* exploits for long-term survival. Rapamycin helps to expose *M. tb* to the host immune system by breaking down these protective niches, facilitating its clearance (Zullo and Lee 2012; Singh and Subbian 2018). Verapamil not only affects bacterial resistance but also influences the host's immune system by decreasing inflammation and enhancing the elimination of infected cells.

Navigating Biological and Clinical Barriers: Repurposing Drugs for *M. tb*

Repurposing drugs for TB involves several biological and clinical challenges. These include off-target effects, toxicity, and the need for optimized dosing regimens. These drugs were not originally designed to target *M. tb*, so they may interact with other cellular pathways or organs unintendedly. Off-target effects can lead to undesirable side effects, limiting the clinical utility of repurposed drugs (Aguilar Diaz et al. 2023; Natsheh et al. 2024).

In repurposing drugs for *M. tb*, dosing regimens often need adjustment to achieve therapeutic efficacy against this pathogen. For example, linezolid is administered at higher doses than usual for TB treatment, while metformin is used at lower doses to enhance host immune responses. These adjustments are crucial to balancing efficacy and minimizing toxicity, highlighting the complexity of repurposing drugs for diseases with unique pathogenic mechanisms. Standard doses may not always suffice due to differences in bacterial physiology and drug penetration into granulomas. Optimization studies are ongoing to balance efficacy and safety.

It is crucial to tackle these concerns to guarantee the safe and effective use of repurposed medications in TB patients (Maitra et al. 2016; Alffenaar et al. 2022; Sharma et al. 2023). One of the primary concerns with repurposed drugs is their potential off-target effects. For example, linezolid, an antibiotic repurposed for MDR-TB, has been associated with significant side effects, including myelosuppression and peripheral neuropathy. These off-target effects arise from the action of linezolid on human mitochondrial protein synthesis, which shares similarities with the biochemical machinery of protein synthesis in bacteria. This highlights the challenge of repurposing drugs with complex mechanisms of action, as their interactions with human biology may lead to adverse side effects (Jaspard et al. 2020; Yuan et al. 2022; Sharma et al. 2023). Toxicity is a major challenge in drug repurposing, particularly for drugs that were originally developed for short-term use or conditions unrelated to chronic infections, such as TB. When repurposed for TB, which requires long-term treatment, these drugs may accumulate in the body and cause toxic effects (Fatima et al. 2021; Johnson et al. 2024). Clofazimine, a drug repurposed from leprosy treatment for TB, has demonstrated efficacy against *M. tb*. However, long-term use is associated with skin discoloration, gastrointestinal disturbances, and cardiotoxicity. These toxic effects can limit patient adherence to treatment and may necessitate discontinuation of the drug, thereby reducing its overall effectiveness (Murashov et al. 2018; Stadler et al. 2023).

Precision in Dosing: Enhancing Repurposed Drug Efficacy Against *M. tb*

The optimization of dosing regimens is essential to ensure their effectiveness against TB. This can be particularly challenging, as the pharmacokinetics and pharmacodynamics of these drugs may significantly differ when used for TB compared with their original indications (Alffenaar et al. 2022). For instance,

metformin, a widely used antidiabetic drug repurposed for TB, may require adjustments in dosing to achieve the desired effects on *M. tb* without compromising glucose control in patients with diabetes. In non-diabetics, metformin has shown potential for enhancing immune responses against *M. tb* without significantly affecting blood glucose levels. It modulates the host's immune pathways, such as activating AMPK and reducing inflammation, which benefits TB treatment, irrespective of diabetic status (Singhal et al. 2014). Additionally, the interaction of repurposed drugs with standard TB therapies must be carefully considered to avoid drug-drug interactions that could reduce treatment efficacy or increase toxicity (Tseng 2018; Sutter et al. 2022). In many cases, clinical trials are needed to determine the optimal dosing regimens of repurposed drugs for TB. However, conducting these trials can be time-consuming and costly, adding to the complexity of the repurposing process (du Cros et al. 2023).

Navigating Regulatory and Economic Hurdles in Repurposing Drugs for *M. tb*

In addition to biological and clinical challenges, drug repurposing for TB faces significant regulatory and economic barriers. This includes issues related to drug approval, patent protection, and economic incentives for pharmaceutical companies. Regulatory approval is a critical hurdle in repurposing drugs for TB (Krishnamurthy et al. 2022). Although repurposed drugs have already been approved for other indications, their use in TB requires new clinical trials to demonstrate their safety and efficacy in the context of this disease. This can be a lengthy and expensive process, particularly for drugs that were originally approved decades ago and may not meet the current regulatory standards (Tiberi et al. 2018b). Moreover, repurposed drugs may require additional regulatory scrutiny if their use in TB requires higher doses, longer treatment durations, or combination therapies with other drugs. Regulatory agencies may demand extensive data on these aspects, thereby further delaying the approval process. Bedaquiline was fast-tracked owing to the urgent need for new TB treatments. Similar fast-tracking is less likely for repurposed drugs unless there is compelling evidence of their efficacy and safety (Fatima et al. 2021; Villa et al. 2024).

Another significant challenge in drug repurposing is patent protection. Many drugs considered for repurposing are off-patent, meaning that pharmaceutical companies may have limited financial incentives to invest in their development. Without the intellectual property rights provided by patents, companies may be reluctant to fund the necessary clinical trials and regulatory submissions, as they may be unable to recoup their investment (Cha et al. 2018; van der Pol et al. 2023). Furthermore, even if a repurposed drug is still under patent, the original patent may not cover its use for TB. In such cases, companies would need to file new patents for TB indications, which can be a complex and uncertain process. The possibility of patent litigation adds another layer of risk, which may deter companies from pursuing drug repurposing for TB (Krishnamurthy et al. 2022; Yadav 2023). A significant obstacle to repurposing drugs for TB is the absence of financial motivation. TB predominantly impacts nations with low and middle incomes, where

the demand for novel treatments may be constrained. Pharmaceutical firms might be reluctant to allocate resources for repurposing drugs targeting TB due to concerns that the potential profits may not offset the expenses involved. In response to this issue, collaborations between public and private entities as well as worldwide health initiatives, have been instrumental in promoting drug repurposing efforts for TB (Mueller-Langer 2013; Zhang et al. 2020). Funding and support for research on repurposed drugs have been provided by entities such as the Global Fund, the WHO, and the Bill & Melinda Gates Foundation. However, sustainable economic incentives are still needed to encourage the involvement of broader pharmaceutical companies in TB drug development. While drug repurposing offers a promising approach to addressing the global TB crisis, it is not without its challenges (Kruk et al. 2018).

Recent Advances and Future Perspectives in Drug Repurposing for TB Emerging Technologies and Approaches

Recent advances in technology and methodology can potentially revolutionize drug repurposing for TB treatment. These innovations can streamline the discovery process, improve the precision of drug targeting, and enhance treatment outcomes (Urbina et al. 2021; Miethke et al. 2021).

CRISPR-Cas9 technology, a powerful tool for gene editing, has been increasingly used in TB research. CRISPR-based screens allow scientists to systematically knock out or modify specific genes in *M. tb* to identify the critical genes involved in bacterial survival and pathogenicity. By identifying these essential genes, researchers can pinpoint potential drugs that repurposed drugs can target. CRISPR screens have been used to identify genes involved in *M. tb* resistance to oxidative stress and antibiotics. Drugs that target these genes could be repurposed to weaken bacterial defenses and improve treatment efficacy. Additionally, CRISPR can be used to study host-pathogen interactions, revealing how *M. tb* manipulates host cells to evade immune responses (Sodani et al. 2023; Ebrahimi et al. 2023). Targeting these interactions with repurposed drugs may lead to new therapeutic strategies.

In drug discovery, particularly for TB repurposing, artificial intelligence and machine learning have made significant strides (Jamal et al. 2020; Stokes et al. 2020). These advanced technologies can process enormous datasets from diverse sources, including chemical libraries, clinical trials, and genomic databases, to identify potential repurposed drugs that exhibit anti-TB properties. The AI-driven approach to drug discovery expedites finding new drug candidates by forecasting drug-target interactions, enhancing drug combinations, and pinpointing compounds with desirable pharmacokinetic and toxicity characteristics. For instance, AI models can anticipate how existing medications interact with *M. tb* proteins, leading to the discovery of new therapeutic targets. Furthermore, AI can be employed to formulate novel drug combinations that boost the effectiveness of repurposed drugs, especially for MDR-TB and XDR-TB cases (Singh et al. 2023b; Visan and Negut 2024).

For decades, TB treatment has relied on combination therapies that utilize multiple drugs with synergistic mechanisms of action. The development of more

effective combination therapies has been facilitated by recent progress in elucidating drug-drug interactions and understanding how repurposed medications function. This improved knowledge has opened up new possibilities for enhancing TB treatment strategies (Larkins-Ford et al. 2022).

For instance, the combination of repurposed drugs, such as verapamil (a calcium channel blocker), with standard TB drugs has shown promise in overcoming drug resistance. Verapamil enhances the intracellular concentration of TB drugs by inhibiting efflux pumps, which *M. tb* uses to expel antibiotics from its cells. This synergistic effect can improve treatment outcomes, particularly in drug-resistant TB cases (Mitini-Nkhoma et al. 2021; Remm et al. 2022). Furthermore, advances in pharmacogenomics and systems biology are helping researchers design more precise combination therapies that minimize toxicity and maximize efficacy. By tailoring drug combinations to the genetic and metabolic profiles of the patient and pathogen, personalized combination therapies could become a reality in TB treatment (Oates and Lopez 2018; Marques et al. 2024).

Future Directions

As the field of drug repurposing for TB continues to evolve, several key trends and future directions have emerged. These include the integration of personalized medicine, the use of innovative clinical trial designs, and the potential for repurposing drugs in novel ways. The future of TB treatment looks promising with the advent of personalized medicine, which customizes therapies based on each patient's unique characteristics. Recent progress in genomics, proteomics, and metabolomics has allowed scientists to gain deeper insights into why individuals respond differently to TB treatments (Ejalonibu et al. 2021; Dartois and Rubin 2022). In the context of drug repurposing, personalized medicine could involve selecting repurposed drugs based on the genetic makeup of the patient or the specific strain of *M. tb* they are infected with. As an illustration, some individuals may respond more favorably to repurposed medications that focus on particular host-pathogen interactions, while others might experience better results from drugs targeting bacterial metabolic processes (Hora et al. 2024). The integration of personalized medicine with drug repurposing could potentially lead to the development of more efficacious and less harmful treatments for TB.

Clinical trials are crucial to validate the effectiveness of repurposed drugs for TB treatment. These trials not only assess the efficacy of individual drugs but also explore novel drug combinations and dosing regimens. Future clinical trials are likely to focus on the optimization of repurposed drugs in combination with existing anti-TB therapies. Innovative trial designs, such as adaptive trials, allow real-time modifications based on interim results and could accelerate the development of new treatment regimens. Additionally, clinical trials may explore the use of repurposed drugs in specific populations, such as patients with MDR-TB or comorbidities such as diabetes.

In addition to conventional drug repurposing, there is increasing attention on repurposing medications for innovative therapeutic strategies, including HDTs and immunomodulatory treatments. Instead of directly attacking the bacterium, HDTs

seek to enhance the host's immune response to TB (Figure 2). Repurposed drugs that modulate the immune system, such as statins and immunomodulatory agents, are being explored for their potential to enhance the body's ability to control TB infection. Another exciting area of research is using repurposed drugs to target latent TB infection. While most current TB treatments focus on active disease, repurposing drugs to prevent the reactivation of latent TB could significantly reduce the global TB burden. Medications that regulate immune responses or suppress *M. tb* dormancy pathways can be adapted for treating latent TB. The area of drug repurposing for TB is experiencing rapid growth, propelled by new technologies such as CRISPR-based screening, artificial intelligence in drug development, and multi-drug therapeutic approaches. These advancements can expedite the identification of novel TB treatments, enhance drug targeting accuracy, and boost treatment efficacy. In the coming years, the incorporation of individualized medicine, current clinical studies, and innovative therapeutic strategies will likely influence the trajectory of drug repurposing for TB management. By addressing existing obstacles and embracing these developments, the worldwide TB community can make significant strides toward eliminating this devastating illness.

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CHAPTER
7

Repurposing Drugs as Anthelmintics: Expanding the Therapeutic Arsenal Against Parasitic Worm Infections

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Introduction

Helminth parasites are a diverse group of worms belonging to phyla Platyhelminthes (flatworms) or Nematoda (roundworms). These parasitic worms infect humans, animals, and plants, causing a diverse array of diseases collectively known as helminthiases. For humans and domestic animals, many helminthiases are of significant public health concern worldwide, particularly in environments with poor sanitation and hygiene, where they disproportionately affect populations in resource-limited areas (Hotez et al. 2008). Helminthiases can lead to chronic morbidity, developmental impairments, and socioeconomic burdens, posing challenges to public health systems and impeding socioeconomic development in endemic regions.

Table 1 shows the helminthiases of most concern worldwide, considering their global impact on human populations. The inclusion of these helminthiases into the neglected tropical diseases (NTDs) list by the World Health Organization (WHO 2025e) reflects their designation as diseases of poverty, requiring urgent and effective government initiatives to mitigate their impact on public health and alleviate the socioeconomic burden they impose.

The complex life cycles of these parasitic worms, coupled with environmental and socioeconomic factors, contribute to their widespread prevalence and persistent challenges for control and elimination efforts. In this scenario, anthelmintic drugs are vital for managing parasitic worm infections and have significantly contributed

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Table 1. Helminthiases included in the WHO's list of neglected tropical diseases (NTDs), their etiological agents, and drugs most currently used for their treatment ⏎

Diseases	Etiological Agents	Drugs Currently in Use
Lymphatic and subcutaneous filariases, incl. elephantiasis, onchocerciasis (river blindness), and dracunculiasis	Filarial nematode worms, including *Wuchereria bancrofti*, *Brugia malayi*, *Brugia timori*, *Onchocerca volvulus*, and *Dracunculus medinensis*	Ivermectin, diethylcarbamazine, and albendazole
Soil-transmitted helminthiases, including ascariasis, strongyloidiasis, trichuriasis, and hookworm diseases (ancylostomiases)	Roundworms (*Ascaris lumbricoides* and *Strongyloides stercoralis*), whipworms (*Trichuris trichiura*), and hookworms (*Necator americanus* and *Ancylostoma duodenale*)	Albendazole, mebendazole, and ivermectin
Intestinal and urogenital schistosomiases	Blood flukes (*Schistosoma mansoni*, *Schistosoma japonicum*, *Schistosoma mekongi*, *Schistosoma guineensis*, *Schistosoma intercalatum*, and *Schistosoma haematobium*)	Praziquantel (for all schistosome species), and oxamniquine (for *S. mansoni*), and metrifonate (for *S. haematobium*)
Taeniases and cysticercoses, including. visceral cysticercosis and neurocysticercosis	Adult and larval (cysticercus) stage of *Taenia solium* (the pork tapeworm)	Taeniasis: praziquantel, albendazole or niclosamide (for taeniasis); and praziquantel and/ or albendazole (for cysticercoses)
Echinococcoses, including alveolar echinococcosis and cystic echinococcosis	Larval (metacestode) stage of tapeworms of the genus *Echinococcus*	Albendazole, and mebendazole
Foodborne trematodiases caused by liver and lung flukes, including clonorchiasis, opistorchiasis, fascioliasis and paragonimiasis	Liver flukes of the genera *Clonorchis*, *Opisthorchis*, and *Fasciola*, and lung flukes of the genus *Paragonimus*	Praziquantel, triclabendazole, niclosamide, and albendazole

to global efforts to control and eliminate helminthiases. However, the emergence of drug resistance, limited efficacy against certain parasite species, and adverse side effects underscore the pressing need to develop better anthelmintic agents.

Unfortunately, the cost of developing new anthelmintic drugs can be prohibitively high and, therefore, may not be of interest to the pharmaceutical industry. *De novo* drug discovery often involves extensive investments in research and development, preclinical and clinical testing, regulatory approvals, and manufacturing processes.

The inherently high failure rates and lengthy timelines associated with traditional drug discovery pipelines further exacerbate the financial burden of developing novel anthelmintics. Moreover, the market for anthelmintic drugs, particularly in low- and middle-income countries, is constrained by factors such as affordability, access to healthcare, and competing public health priorities, which pose challenges to recouping investment costs.

In contrast to *de novo* drug discovery, drug repurposing, also known as drug repositioning or drug reprofiling, is a more promising strategy to provide novel anthelmintic drugs. It offers a cost-effective alternative by leveraging existing drugs with established safety profiles and pharmacokinetic properties. By repurposing drugs that have already undergone rigorous testing for other indications, researchers can expedite the drug development process, significantly reduce costs, and mitigate the risks associated with *de novo* drug discovery. This strategy allows for the rapid identification of promising anthelmintic candidates, accelerating their translation from preclinical studies to clinical trials and ultimately facilitating their deployment to treat helminth infections.

In this chapter, we will begin by examining the key attributes of currently utilized anthelmintic drugs and their associated limitations. Then, we will present various strategies available for drug repositioning as anthelmintics. Finally, we will showcase promising examples of efforts to provide novel anthelmintic drugs based on drug repositioning strategies.

Drugs Currently in Use for the Treatment of the Main Human Helminthiases

This section briefly presents the helminthiases in the WHO's list of NTDs and the currently available drugs used for their treatment. These drugs, also listed in Table 1, have been meticulously selected based on their efficacy, safety profiles, and suitability for resource-limited settings, playing crucial roles in addressing the burden of helminth infections among vulnerable populations worldwide. However, as we will also point out, they all present limitations, justifying ongoing efforts for drug repositioning as novel anthelmintics.

Drugs in Use for the Treatment of Filariases

Filariases are a collection of diseases caused by filarial nematodes of the superfamily Filarioidea. The most relevant human filariases are the lymphatic filariases caused by species such as *Wuchereria bancrofti*, *Brugia malayi*, and *Brugia timori* (WHO 2025d), and two of the so-called subcutaneous filariases, namely onchocerciasis, caused by *Onchocerca volvulus* (WHO 2025f), and dracunculiasis, caused by *Dracunculus medinensis* (the Guinea worm) (WHO 2025a). Lymphatic filariases are transmitted by mosquitoes, while onchocerciasis and dracunculiasis are respectively transmitted by black flies and small crustaceans (copepods, also called water fleas).

In lymphatic filariases, the lymphatic system is compromised by worm infection and growth. In chronic cases, an affected individual may develop elephantiasis, a condition where parts of the body swell to massive proportions, causing pain, severe

disability, and social stigma. Onchocerciasis, or river blindness, is a disease affecting the eyes and skin. Its symptoms include severe itching, disfiguring skin conditions, and visual impairment, which can lead to permanent blindness. Dracunculiasis, in turn, is typically asymptomatic for the first year of infection. Debilitating symptoms appear when the worm erupts through the skin, usually on the lower leg, including intense itching and burning pain at the site of the lesion. Besides, urticaria, erythema, dyspnea, vomiting, and pruritus are also common, reflecting allergic reactions to worm antigens.

Regardless of the type of filariasis, there are currently only three drugs with widely recommended use for treatment, namely ivermectin, diethylcarbamazine, and albendazole (Freitas et al. 2024). In the cases of lymphatic filariases and onchocerciasis, WHO has also recommended mass administration of different combinations of these drugs as a preventive chemotherapy strategy for at-risk populations as a whole (Bockarie et al. 2013). This is expected to reduce the reservoirs of infection in the long term. The effectiveness of these three drugs against filariae is due to different mechanisms of action (see the Drugbank database (Knox et al. 2024) and the Antimicrobe database (Burdette et al. 2006) for more details). Ivermectin, a macrocyclic lactone, binds selectively with high affinity to glutamate-gated chloride ion channels and acts as an agonist of the gamma-aminobutyric acid (GABA) neurotransmitter, impairing invertebrate muscle and nerve cells, ultimately leading to paralysis and death of the parasites. In *O. volvulus*, it may also impair normal larval development. Diethylcarbamazine is thought to sensitize early-stage larvae (microfilariae) to phagocytosis in a mechanism of action involving prostaglandin metabolism in microfilariae and/or host endothelial cells. Finally, albendazole binds to tubulin, thus inhibiting its polymerization or assembly into microtubules. With that, it causes degenerative alterations in the tegument and intestinal cells of the parasites, ultimately leading to immobilization and death. However, the effectiveness of albendazole for treating filariases has been under debate. Indeed, there is good evidence that albendazole makes little difference in clearing microfilaraemia or adult filarial worms in the 12 months post-treatment, either in trials with albendazole alone or in combination with ivermectin or diethylcarbamazine (Macfarlane et al. 2019).

Despite being the primary drugs for filariasis control, ivermectin, diethylcarbamazine, and albendazole have notable limitations (Katiyar and Singh 2011). A significant challenge is their incomplete efficacy against adult filarial worms (macrofilariae), leading to persistent infections and continued transmission. These drugs effectively target microfilariae but have limited effects on macrofilariae, demanding repeated treatments over several years to achieve long-term control. Additionally, the emergence of drug resistance, as already documented for ivermectin and albendazole (Cobo 2016; Collins et al. 2024), poses a significant threat to the sustainability of these treatments. The side effects associated with the available antifilarial drugs, including severe inflammatory reactions to dying microfilariae, can also complicate treatment adherence and completion (CDC 2024). Furthermore, the logistical and financial challenges of implementing mass drug administration programs in endemic regions, especially in areas with limited resources, often hinder the comprehensive coverage needed to break the transmission cycle (Dinesh et al. 2023). These limitations underscore the urgent need for innovative strategies to

improve the control and eventual eradication of filariases, including developing new therapeutic agents.

Drugs in Use for the Treatment of Soil-transmitted Helminthiases

Soil-transmitted helminthiases are a group of parasitic infections caused by nematode helminths that are transmitted through contact with soil contaminated with human feces carrying the worms' eggs (WHO 2025h). The primary soil-transmitted helminths include the roundworms *Ascaris lumbricoides* and *Strongyloides stercoralis*, the whipworm *Trichuris trichiura*, and the hookworms *Ancylostoma duodenale* and *Necator americanus*. These infections are prevalent in regions with poor sanitation and hygiene practices, particularly in tropical and subtropical areas. Together, these parasites significantly impact the health, development, and economic productivity of affected populations, particularly children. *A. lumbricides* infection (ascariasis) is the most common human helminthiasis worldwide. It may impair nutrient absorption, leading to malnutrition and stunted growth. Heavy infections may even cause intestinal blockage. *S. stercoralis* infection (strongyloidiasis) causes abdominal pain, diarrhea, rash, pulmonary symptoms (cough and wheezing), and eosinophilia. *T. trichiura* infection (trichuriasis), in turn, is often asymptomatic, but heavy infections can cause gastrointestinal problems, including abdominal pain and diarrhea. Finally, infections caused by hookworms *A. duodenale* or *N. americanus* (also known as ancylostomiases) can cause from itchy rashes to respiratory and gastrointestinal problems. Eventually, they also cause iron-deficiency anemia and protein loss due to the worms' blood-feeding behavior.

The most commonly used drugs for the treatment and preventive chemotherapy of soil-transmitted helminthiases are albendazole and mebendazole (Keiser 2023), which are both effective against *A. lumbricoides*, *T. trichiura*, *A. duodenale*, and *N. americanus*. These two benzimidazoles inhibit the production of microtubules in the intestinal cells of parasites, impairing glucose uptake and disrupting digestive and reproductive functions (Lacey 1990). This interruption leads to the immobilization of the worm, hinders egg production, and ultimately causes its death. Ivermectin, whose mechanism of action was previously mentioned, has also been used to treat soil-transmitted helminthiases. This broad-spectrum parasitic drug has shown high efficacy against *S. stercoralis* and *A. lumbricoides*, moderate efficacy against *T. trichiura*, and poor efficacy against *A. duodenale* and *N. americanus* (Le et al. 2024).

Albendazole, mebendazole, and ivermectin are commonly used, either in combination or alone, in mass drug administration programs for the control and prevention of soil-transmitted helminthiases worldwide (Moser et al. 2019; Lebu et al. 2023). However, these treatments have notable limitations. Resistance to benzimidazoles, linked to mutations in the β-tubulin protein of the worms, has been documented, reducing the efficacy of these drugs (Ng'etich et al. 2024). Additionally, while albendazole and mebendazole are effective for treating intestinal helminthic infections, their effectiveness is significantly diminished against extraintestinal forms of the parasites due to poor absorption and low systemic bioavailability. Moreover, the efficacy of ivermectin against strongyloidiasis has reportedly declined in some regions due to its prolonged use in mass treatment programs, leading to concerns about the potential development of resistance (Sengthong et al. 2024).

Drugs in Use for the Treatment of Schistosomiases

Schistosomiases are helminthiases caused by parasitic platyhelminth trematode worms of the genus *Schistosoma*, commonly known as blood flukes. There are two major forms of schistosomiasis, intestinal and urogenital, caused by different blood fluke species (WHO 2025g). Intestinal schistosomiases result from infections with *Schistosoma mansoni*, *Schistosoma japonicum*, *Schistosoma mekongi*, *Schistosoma guineensis*, or *Schistosoma intercalatum*. Urogenital schistosomiasis is caused by *Schistosoma haematobium*. These infections occur when people come into contact with freshwater contaminated with the larvae of the parasites released by infected snails. Once inside the human host, the parasites mature and reproduce, leading to a range of symptoms that can include abdominal pain, diarrhea, and blood in the urine or stool. Chronic infection can cause severe complications such as liver and kidney damage, bladder cancer, and growth retardation in children. Schistosomiases pose significant public health challenges in many tropical and subtropical regions in Africa, Asia, and South and Central America.

Chemotherapeutic treatment for schistosomiasis has primarily relied on a single drug, praziquantel, for the past five decades (Waechtler et al. 2023). Praziquantel is effective against all species of schistosomes, making it the cornerstone of schistosomiasis control programs. In contrast, other drugs initially used, such as oxamniquine and metrifonate, have more limited efficacies (Cheuka 2021; Condeng et al. 2024). Oxamniquine specifically targets *S. mansoni*, while metrifonate is effective exclusively against *S. haematobium*. The primary mechanism of action of oxamniquine involves the ATP-dependent generation of an intermediate that alkylates essential macromolecules, including DNA (Pica-Mattoccia and Cioli 1985). On the other hand, metrifonate is metabolized in byproducts that inhibit acetylcholinesterase in schistosome muscles, causing irreversible paralysis of the worm (Holmstedt et al. 1978). For praziquantel, the parasite target is a transient receptor potential ion channel of the melastatin family ($TRPM_{PZQ}$), which, upon interaction with the drug, causes Ca^{2+} influx and spastic paralysis of adult worms and rapid vacuolization of the worm surface (Park et al. 2019).

Despite its widespread use for schistosomiasis treatment, praziquantel has certain limitations (Condeng et al. 2024). It is ineffective against immature worms, which can result in re-infections and treatment failure in endemic areas. Additionally, prolonged and extensive use of praziquantel may lead to resistance and tolerance, potentially reducing its efficacy over time (Ezebuo and Uzochukwu 2022). Similarly, oxamniquine and metrifonate have limitations beyond their narrow spectrum of activity. Both drugs have also faced treatment failures due to the development of resistance or tolerance in some cases (Bruce et al. 1987). These challenges highlight the necessity for ongoing research for new therapeutic drugs to ensure effective and sustainable schistosomiasis control.

Drugs in Use for the Treatment of Taeniases and Cysticercoses

Human taeniasis and cysticercoses are diseases caused by platyhelminth cestodes of the genus *Taenia*, including *Taenia solium* (the pork tapeworm), *Taenia saginata* (the beef tapeworm), and *Taenia asiatica* (Ito and Budke 2021). WHO includes only

T. solium taeniasis and cysticercoses in its NTDs' list (Table 1) (WHO 2025i), as the ones of more impact on human health. In this section, we will also focus on *T. solium* infections.

Taeniasis occurs when humans consume undercooked or raw pork infected by *T. solium* larvae, thus developing adult tapeworms in the intestines (Okello and Thomas 2017). Symptoms of taeniasis are often mild but may include digestive issues such as abdominal pain, nausea, and weight loss. Cysticercoses, on the other hand, are more severe conditions resulting from the ingestion of *T. solium* eggs, typically through contaminated food or water (Hossain et al. 2023). The ingested eggs hatch into larvae, which migrate through the bloodstream and form cysts (cysticerci) in various tissues. Cysticercoses are categorized based on the site of infection, which includes muscular cysticercosis, subcutaneous cysticercosis, ocular cysticercosis, cardiac cysticercosis, and neurocysticercosis. Symptoms vary depending on the infected tissue or organ. Neurocysticercosis is the more threatening form of the disease, as it involves the development of *T. solium* cysts in the central nervous system and can cause severe neurological symptoms, including seizures and headaches, and may be fatal in extreme cases.

Adult worm infections in taeniasis cases can be easily prevented or eliminated by chemotherapy using albendazole, praziquantel, or niclosamide (Pan American Health Organization 2021). The mechanisms of action for albendazole and praziquantel have been previously discussed. Niclosamide inhibits oxidative phosphorylation and impairs the parasite's glucose uptake, ultimately leading to its death from lack of energy (Ryan 2018).

Cysticercoses, on the other hand, are more challenging to treat due to the cystic nature of the cysticerci, which limits drug access and makes surgical removal often too risky, especially in cases of neurocysticercosis or ocular involvement (Garg et al. 2024). Chemotherapeutic treatment typically involves praziquantel and/or albendazole, with drug dosages and treatment durations varying significantly based on the number, size, location, and developmental stage of the cysticerci, with immature cysts being less responsive to treatment (Hamamoto Filho et al. 2022). High doses and prolonged treatments are often necessary, leading to side effects such as gastrointestinal disturbances, liver toxicity, and bone marrow suppression. The extensive use of praziquantel and albendazole may also promote the emergence of resistance. Furthermore, the destruction of cysts can trigger inflammatory responses, necessitating aggressive supportive therapy with corticosteroids and/or anti-epileptic drugs.

Drugs in Use for the Treatment of Echinococcoses

Echinococcoses, also called hydatid diseases, have platyhelminth cestodes of the genus *Echinococcus* as their etiological agents. There are two main forms of echinococcosis: alveolar echinococcosis (which is caused by *Echinococcus multilocularis*) and cystic echinococcosis (which is caused by *Echinococcus granulosus* sensu lato (s. l.), a complex of cryptic species) (Nakao et al. 2013). Both alveolar and cystic echinococcoses are significant public health concerns and have been included in the WHO's list of NTDs (WHO 2025b). These diseases are characterized by the growth of larvae (metacestodes), which form multilocular

cysts in the case of alveolar echinococcosis or unilocular cysts in the case of cystic echinococcosis. The parasites primarily affect the liver or lungs, leading to long asymptomatic incubation periods followed by severe clinical manifestations due to invasive lesions in the affected organs.

Echinococcoses are prevalent worldwide, with endemic and hyperendemic regions on all inhabited continents (Deplazes et al. 2017). Alveolar echinococcosis is primarily distributed in the northern hemisphere, while cystic echinococcosis has a global distribution. Surgical removal of Echinococcus metacestodes is often difficult and risky. Consequently, chemotherapy is the most common form of treatment for both alveolar and cystic echinococcosis (Eckert and Thompson 2017). Prolonged treatments with high oral doses of benzimidazoles have been used, with albendazole and mebendazole (addressed in previous sections) being the drugs of choice. Despite being highly effective against adult worms, praziquantel has little effect on the pathogenic metacestodes (Olson et al. 2012) due to its targeting of voltage-dependent calcium channels, which are not expressed in the larval forms (Koziol and Brehm 2015).

It is also important to note that chemotherapy with benzimidazoles is often only partially effective and rarely curative. Additionally, the high doses and long treatments can increase the occurrence and severity of side effects. Moreover, repeated treatments with a single or few drugs raise concerns about the emerging spread of drug resistance (Salinas et al. 2017; Wen et al. 2019).

Praziquantel, which is highly active against adult worms, has little effect on the pathogenic alveolar or cystic larval forms (metacestodes) of the parasites. This is because praziquantel targets voltage-dependent calcium channels, which are not expressed in metacestodes (Koziol and Brehm 2015).

Drugs in Use for the Treatment of Foodborne Trematodiases

Foodborne trematodiases constitute a group of diseases caused by platyhelminth trematodes (flukes) (Keiser and Utzinger 2009). The infections caused by liver flukes, such as clonorchiasis (caused by *Clonorchis sinensis*), opisthorchiasis (caused by *Opisthorchis viverrini* and *Opisthorchis felineus*), and fascioliasis (caused by *Fasciola hepatica* and *Fasciola gigantica*), as well as paragonimiasis (caused by lung flukes of the genus *Paragonimus*), are of major significant public health concern. As such, these diseases are recognized in the WHO's list of NTDs (WHO 2025c). Humans acquire infections with foodborne trematodes by eating raw, undercooked, or poorly processed food contaminated with the infective larval stages (metacercariae) of the parasites. Common sources of infection include freshwater fish, crustaceans (such as crabs and crayfish), and aquatic plants. These trematode infections are distributed worldwide but occur most commonly in Asia (Tidman et al. 2023). Together, they cause an estimated 2 million life years lost to disability and death worldwide every year (Chai and Jung 2022).

Although foodborne liver and lung trematodiases may initially be asymptomatic, they can cause severe pathologies in advanced cases (Keiser and Utzinger 2009). The major pathogenesis in liver trematode infections includes focal hemorrhage, inflammatory reactions, and necrotic lesions. More serious complications, ranging from cholangitis and cholecystitis to liver cirrhosis and cholangiocarcinoma, may

also develop. The most common clinical manifestations of lung fluke infections are cough, bloody sputum, and bronchiectasis. However, these flukes can also migrate to extrapulmonary sites, such as the brain, spinal cord, skin, subcutaneous tissues, and abdominal organs.

The treatment of liver and lung foodborne trematodiases primarily relies on the use of praziquantel, albendazole, and triclabendazole as flukicides (Chai and Jung 2022; Tidman et al. 2023). The mechanisms of action for albendazole and praziquantel have been discussed in previous sections. Triclabendazole, similar to albendazole and other benzimidazoles, binds to β-tubulin and inhibits the formation of microtubules (Drugbank 2025). This and other indirect effects affect the motility, tegument integrity, and reproduction of the parasites. Praziquantel or albendazole are recommended for treating clonorchiasis and opisthorchiasis, while triclabendazole is recommended for fascioliasis. Both praziquantel and triclabendazole can be used to treat paragonimiasis. Triclabendazole has been gaining recognition as the first-choice flukicide. However, it is not registered in all countries, and there have been reports of drug resistance, adverse reactions, and difficulties in obtaining the medication (Fairweather et al. 2020; Marcos et al. 2021).

Different Strategies for Drug Repurposing as Novel Anthelmintics

This section explores current strategies that can be utilized for drug repurposing as anthelmintics. A brief description of each strategy is provided, highlighting its strengths and potential use in drug repurposing and developing more effective treatments for human parasitic worm infections.

Phenotypic Screening

Phenotypic screening involves systematically evaluating existing drugs, usually *in vitro*, to determine their capacity to induce desired phenotypic changes in parasitic worms (Herath et al. 2022). By focusing on observable phenotypes, such as parasite morphology, motility, reproduction, and viability, phenotypic screening approaches offer a holistic perspective on drug efficacy and allow for identifying novel anthelmintics with diverse mechanisms of action.

A phenotypic screening can be conducted through small-scale (or low-throughput) assays, where the effects of tens to hundreds of known drugs on worm cultures are assessed in multiwell plates. While these low-throughput assays are more accessible for low-budget research, they are also more time-consuming. Each control or drug-exposed worm sample must be assessed at regular intervals, ranging from hours to days, under a microscope or in instruments like micro-motility meters/recorders or respirometers.

Alternatively, medium- or high-throughput screening can also be employed. These large-scale assays use automated or semi-automated platforms that include devices that allow large-scale phenotypic recording and data analysis. Detection instruments that can be integrated into these platforms include spectrophotometers, spectrofluorometers, fluorescence microplate readers, cytometers, multi-channel

isothermal microcalorimeters, and high-resolution imaging systems, to name a few. The technologies developed so far enable the rapid screening of a much wider range of drugs (up to thousands) and the assessment of several phenotypes, including morphology, growth, motility, tissue damage, egg hatching, ATP production, or enzyme activity. Recent advances in high-content imaging and machine learning have also enhanced the detection of subtle cryptic phenotypes (Zamanian and Chan 2021). However, it is important to note that access to extensive drug libraries and technologies amenable to medium- to high-throughput assays can be quite costly.

Protocols and instrumentation for phenotypic screening have been developed for various species of worms (Herath et al. 2022). Most of them have been developed for nematodes of veterinary importance, including species of the genera *Ancylostoma*, *Ascaris*, *Brugia*, and *Trichuris*; despite being a free-living worm, the nematode *Caenorhabditis elegans* has also been extensively utilized as a model system in the search for anthelmintic drugs effective against parasitic nematodes (Hahnel et al. 2020). Other assays have been developed for trematodes (flukes), including species of *Schistosoma* (Ravaynia et al. 2022) and *Fasciola* (Keiser et al. 2013), as well as for cestodes (tapeworms) of the genus *Echinococcus* (Kaethner et al. 2023).

Computational Screening

Computational screening is a powerful approach that utilizes bioinformatics, chemoinformatics, and computational modeling techniques to identify potential drug candidates with anthelmintic activity. By analyzing extensive databases of chemical compounds and biological targets, researchers can predict the likelihood of existing drugs exhibiting efficacy against parasitic worms based on their molecular structures and pharmacological properties (Gan et al. 2023). Virtual screening methods, including molecular docking (Muhammed and Aki-Yalcin 2022) and ligand-based similarity searches (Yan et al. 2016), enable the identification of candidate drugs that can interact with specific molecular targets in parasitic worms, providing a rational foundation for further experimental validation.

The field of computational screening is rapidly evolving, with ongoing advancements in molecular docking and structure-based modeling (Rebehmed et al. 2022) and the incorporation of artificial intelligence techniques (Moreira-Filho et al. 2021; Wiwanitmkit and Wiwanitkit 2024). These developments enhance the accuracy and efficiency of the screening process, facilitating the discovery of potential anthelmintic drugs.

An additional noteworthy trend in the field is the emergence of open-science approaches in virtual screening strategies for drug development and repurposing (Partridge et al. 2020). This entails conducting collaborative prospecting work among research groups worldwide, utilizing publicly available databases and bioinformatics tools, and sharing results. This collaborative environment is expected to accelerate the drug discovery process and create new opportunities for drug repurposing.

Target-based Approaches

Target-based approaches for helminth drug discovery (or repurposing) focus on identifying and validating specific molecular targets in parasitic worms that are

crucial for their survival or reproduction (Kulkarni et al. 2023). By unraveling the molecular pathways and biological processes involved in parasite biology, researchers can pinpoint targets for anthelmintic intervention that can be targeted by compounds already approved for use in humans.

Target-based drug repositioning strategies typically include screening existing drugs against known or predicted parasite targets to leverage similarities in target structures or functions between parasites and other organisms (Sprague et al. 2024; Tan et al. 2024). The identification of potential druggable targets is greatly facilitated by whole-genome sequencing of various parasitic helminths, followed by post-genomic functional 'omics' approaches, such as transcriptomics, proteomics, and metabolomics (Preston et al. 2016; Doyle 2022).

Network-based Approaches

Network-based approaches integrate systems biology and other computational tools to comprehensively understand the complex interactions between drugs, targets, and diseases (Ko 2020). By applying systems biology principles, researchers can construct network models of drug-target interactions and biological pathways relevant to helminth biology (Marchat et al. 2023). This enables the identification of potential drug repurposing opportunities for treating parasitic worm infections based on network connectivity and pathway cross-talk. One key aspect of network-based approaches is integrating diverse "omics" data, such as genomics, transcriptomics, proteomics, and metabolomics. By analyzing these datasets, researchers can identify differentially expressed genes, proteins, or metabolites associated with helminthic infections. These differential expression patterns enable the identification of key biological pathways and networks of gene co-expression or protein-protein interactions (PPI) that are dysregulated in helminthic infections, providing insights into potential targets for drug intervention.

In addition to "omics" data integration, network-based approaches also utilize computational modeling techniques to simulate and predict the effects of drug-target interactions (Jenner et al. 2020). Computational models, such as kinetic models or network-based models, can be used to simulate the behavior of biological systems and predict the response of parasites to drug treatments. These models can help identify potential drug candidates for repurposing by assessing their impact on specific molecular targets or pathways involved in helminthic infections.

A major advantage of network-based approaches is their ability to capture the complexity and dynamics of biological systems. Helminthic infections involve intricate host-parasite interactions, and the response to drug treatments can vary depending on the stage of infection, host immune response, and other aspects of parasite biology. Network-based approaches can capture these complexities and provide a more comprehensive understanding of the mechanisms of action of potential anthelmintics. Moreover, network-based approaches can facilitate the identification of drug combinations that exhibit synergistic effects. By analyzing the interactions between multiple drugs and their targets, researchers can identify combinations that enhance the efficacy of anthelmintic treatments. This combinatorial approach can help overcome drug resistance and improve the efficacy of existing drugs.

For helminthiases, network-based approaches for drug discovery or repurposing are still an emergent strategy. Indeed, PPI mapping is still limited to a few helminth parasite species. Among the so far available examples are gene co-expression or PPI partial networks constructed for filarial nematodes (Wangwiwatsin et al. 2023), the trematode *C. sinensis* (Yoo et al. 2020), and the cestodes *E. granulosus* (Paludo et al. 2015; Wang et al. 2023; Pereira et al. 2023) and *T. solium* (Cui et al. 2021). To provide robust databases for dependable network analyses, many more gene co-expression and protein-protein interaction (PPI) datasets from parasitic helminths will be necessary. In the meantime, the free-living nematode *C. elegans* stands as the only helminth model with a more comprehensive gene co-expression and PPI network available (Wang et al. 2023). In the foreseeable future, however, network-based approaches are expected to greatly contribute to identifying druggable targets, elucidating drug mechanisms of action, and discovering drug candidates that can be repurposed as anthelmintics.

Recent Advances in Drug Repurposing for Use as Anthelmintics

In this section, we discuss illustrative examples of recent efforts in repurposing drugs to treat major human helminthiases. The repurposed drugs and their approved and potential applications are outlined in Table 2. Furthermore, we provide detailed descriptions for each group of helminthiases in the subsequent subsections.

Repurposable Drugs for the Treatment of Filariases

As a common characteristic in their biology, most parasitic filarial nematodes of medical relevance, including the ones discussed in this chapter, have evolved a mutually beneficial symbiotic relationship with intracellular bacteria of the genus *Wolbachia* (Taylor et al. 2013). As intracellular endosymbionts, these bacteria are essential for the embryogenesis and survival of these nematodes. Consequently, numerous efforts in drug repurposing have focused on targeting the bacterium rather than directly aiming at the filarial worms.

Notable examples of antibacterial drugs repurposed for antifilarial treatment include doxycycline (a tetracycline) and rifamycin (rifampicine). Doxycycline treatment leads to the permanent sterilization of female worms and sustained loss of microfilariae, ultimately leading to the demise of the adult worms (Taylor et al. 2013). However, the necessity of prolonged treatment courses (lasting 4 to 6 weeks) and the known contraindications of doxycycline for pregnant or breastfeeding women and children under 9 years old hinder its widespread use in mass drug administration control programs (Ehrens et al. 2022).

In the case of rifampicine, its use in antifilarial treatment has been focused on developing a shorter treatment course suitable for mass drug administration (Fordjour and Kwarteng 2022). Studies conducted thus far have shown that high-dose rifampicin has the potential to reduce the duration of treatment for human filariasis to 1–2 weeks. Currently, a Phase II clinical trial is underway to investigate the effectiveness of high-dose rifampicin in treating onchocerciasis (Gokool et al. 2024).

Table 2. Drugs under assessment for repurposing as anthelmintics ⏎

Drug	Current Treatments in Use	Repurposed for Treating
Amphotericin B	Fungal infections	Echinococcoses
Artemisinins	Malaria	Schistosomiases, echinococcoses, and foodborne trematodiases
Auranofin	Rheumatoid arthritis	Filariases (anti-filariae)
Doxycycline	Bacterial infections	Filariases (anti-*Wolbachia*)
Emodepside	Gastrointestinal nematode infections in domestic animals	Filariases (anti-filariae) and soil-transmitted helminthiases
Flubendazole	Gastrointestinal nematode infections in domestic animals	Filariases (anti-filariae)
Halofuginone	Malaria and other protozoal infections	Filariases (anti-filariae)
Histone deacetylase inhibitors	Hematological cancers	Schistosomiases, taeniases, and cysticercoses
Hydroxyurea	Cancer	Echinococcoses
Ivermectin	Nematode infections	Echinococcoses
Mefloquines	Malaria	Schistosomiases, echinococcoses, and foodborne trematodiases
Miltefosine	Breast cancer metastasis	Schistosomiases
Nifuroxazide	Bacterial infections	Schistosomiases
Nilotinib	Chronic hepatitis C	Filariases (anti-*Wolbachia*)
Nitazoxanide	Protozoal infections	Echinococcoses, and foodborne trematodiases
Oxantel pamoate	Gastrointestinal worm infections in cats and dogs	Soil-transmitted helminthiases
Oxbendazole	Gastrointestinal nematode infections in domestic animals	Filariases (anti-filariae), and foodborne trematodiases
Paritaprevir	Leukemia	Filariases (anti-*Wolbachia*)
Quinazoline derivatives	Cancer and bacterial infections	Foodborne trematodiases
Rifamycin	Bacterial infections	Filariases (anti-*Wolbachia*)
Robenidines	Malaria	Schistosomiases
Tamoxifen	Estradiol-dependent breast cancers	Taeniases and cysticercoses
Tribendimidine	Soil-transmitted helminthiases	Taeniases and cysticercoses, and foodborne trematodiases

In the repurposing of drugs to target filarial worms, priority is given to those that are effective against the adult stage of the worms (macrofilariae), as the primary drugs for filariasis control (ivermectin, diethylcarbamazine, and albendazole) are only effective against the larvae (microfilariae). Toward this goal, efforts have been made, for instance, to repurpose flubendazole and oxbendazole, two benzimidazoles of veterinary use against gastrointestinal nematode infections, as macrofilaricides. Flubendazole parenteral administration has long been known for its efficacy as macrofilaricidal in preclinical models (Geary et al. 2019). Ongoing efforts are focused on developing new formulations of flubendazole with high oral bioavailability, which would be more suitable for treating human filariasis. Oxfendazole, on the other hand, has shown potential for treating lymphatic filariasis and onchocerciasis by effectively killing macrofilariae without causing adverse reactions typically associated with drug-induced microfilariae killing (Hübner et al. 2020).

Two additional drugs being repurposed as direct-acting macrofilaricidal are emodepside and auranofin. Emodepside, a member of the cyclooctadepsipeptide class of drugs, is commonly employed in veterinary medicine to treat parasitic gastrointestinal nematodes such as roundworms, hookworms, and Strongyloides. Phase 2 clinical studies are ongoing to assess emodepside for treating *O. volvulus*-infected individuals (Pfarr et al. 2023). Auranofin [2,3,4,6-tetra-O-acetyl-1-thio-beta-D-glucopyranosato-S (triethylphosphine) gold] is a drug approved by the U.S. Food and Drug Administration (FDA) to treat rheumatoid arthritis in humans (Bulman et al. 2015). It is an irreversible inhibitor of glutathione and thioredoxin reductases and thus efficiently kills adult filarial worms, as well as other helminth and protozoan parasites (Feng et al. 2020).

New possibilities for drug repurposing against filariases are also emerging from recent comparative genomic studies, which allow the identification of druggable targets and repurposable drugs. An illustrative example is that of aminoacyl tRNA synthetases, which were identified and compared among humans infecting helminths (Goel et al. 2019). As a result, halofuginone, an antiprotozoan drug, has been identified as a powerful inhibitor of *Onchocerca volvulus* prolyl tRNA synthetase, showcasing its potential as a repurposable drug in the treatment of filariases.

In addition to the previous examples, *in silico* drug repurposing approaches have identified new potential drugs with anti-filarial properties (Kwarteng et al. 2021; Biney et al. 2023). This is the case of nilotinib and paritaprevir, which are FDA-approved medications for treating chronic hepatitis C and leukemia, respectively. These drugs have been predicted to inhibit *Wolbachia* enzymes in the heme biosynthesis pathway, which is crucial for the bacterial symbiotic relationship with filarial worms (Kwarteng et al. 2021). Therefore, nilotinib and paritaprevir show promise as potential candidates for repurposing in the treatment of filarial infections.

Repurposable Drugs for the Treatment of Soil-transmitted Helminthiases

As previously mentioned, the control of soil-transmitted helminthiases primarily relies on preventive chemotherapy using albendazole and mebendazole. However, due to the potential development of drug resistance and limitations in their therapeutic effectiveness, there have been efforts to explore alternative anthelminthic

options, including drug repurposing (Keiser 2023). Currently, two primary candidates for repurposing in treating soil-transmitted helminthiases are oxantel pamoate and emodepside.

Oxantel pamoate is a derivative of tetrahydropyrimidine that has been used in veterinary medicine for several decades in non-rodent species (Palmeirim et al. 2021). Recently, there has been renewed interest in repurposing it for human use due to its high efficacy against human *T. trichiura* infections, although it has limited effectiveness against *A. lumbricoides* and hookworm infections. Oxantel pamoate works by binding to the nicotinic acetylcholine receptor of the parasite in the human gastrointestinal tract, leading to paralysis and expulsion of the worm. Currently, it is only approved and available for human use in certain countries in South America and Asia. However, efforts are underway in Europe to register the drug for treating *T. trichiura* infections at a stringent regulatory authority (Specht and Keiser 2023).

Emodepside, whose repurposing as the macrofilaricidal drug was addressed earlier, is also a leading therapeutic candidate for soil-transmitted helminth infection (Keiser 2023). Preclinical studies have demonstrated the drug's remarkable efficacy against nematode species commonly used as laboratory models, such as *Trichuris muris, Ancylostoma ceylanicum, Necator americanus*, and *Strongyloides ratti* (Karpstein et al. 2019). Furthermore, recent Phase 2a clinical trials have provided evidence of emodepside effectiveness and safety in treating human *T. trichiura* and hookworm infections (Mrimi et al. 2023).

Repurposable Drugs for the Treatment of Schistosomiases

Decades of relying on praziquantel as the primary treatment for schistosomiasis highlight the need for ongoing research to identify effective alternative treatments and minimize the risk of developing resistance. In line with this, the exploration of drug repurposing has yielded promising outcomes in the quest for innovative therapeutic solutions for schistosomiasis.

Notable developments in drug repurposing against schistosomiases and other trematode infections have focused on utilizing antimalarial drugs. The rationale behind this approach lies in the fact that the parasites responsible for causing malaria, such as *Plasmodium* spp., as well as *Schistosome* spp. and other trematodes, rely on blood as their primary source of nutrients. As a result, they share similar heme-detoxification pathways (Xiao and Sun 2017). Promising examples of antimalarial drugs repurposed as anti-schistosomal drugs are artemisinin and mefloquine.

Artemisinin is the active component derived from the *Artemisia annua* plant, also known as quinghao, which has been used in traditional Chinese medicine for centuries. The antimalarial properties of artemisinin were established in the 1970s, and its use as an anthelmintic, either in its natural form or as semi-synthetic and synthetic derivatives, began in the early 21st century (Saeed et al. 2016). Studies have demonstrated that artemisinins are highly effective against juvenile schistosome infections and moderately effective against adult infections, making them particularly suitable as prophylactic treatments. Additionally, they have shown synergistic effects when combined with praziquantel, suggesting their potential use in combination therapy. Ongoing clinical trials are being conducted to evaluate the efficacy and safety of praziquantel plus artemisinin-based combinations in treating *S. mansoni*

infections (Obonyo et al. 2023). These trials aim to assess further the potential of this treatment approach and its impact on combating schistosomiasis.

Mefloquine, on the other hand, is an arylaminoalcohol antimalarial compound that emerged as a potential anti-schistosomal drug in 2008 (Xiao 2013). Not only mefloquine but also several related arylmethanols have demonstrated significant anti-schistosomal properties (Ingram et al. 2012). These compounds are effective against all three major species of *Schistosoma* that infect humans (*S. mansoni, S. japonicum,* and *S. haematobium*). Additionally, they exhibit equal efficacy against both the juvenile and adult stages of the parasites, a characteristic that is not shared by praziquantel or artemisinins. Furthermore, mefloquine, in combination with artesunate (an artemisinin derivative), has shown synergetic effects against schistosomiasis in clinical trials (Roucher et al. 2021; Bottieau et al. 2024). This drug combination is a WHO-recommended therapy for malaria treatment and already has a well-established safety profile. Therefore, it holds promise as a valuable candidate for repurposing against schistosomiasis.

Researchers have also investigated the repurposing of antimalarial drugs to treat schistosomiasis through chemical modification strategies. For example, derivatives of antimalarials like mefloquine (D'Orchymont et al. 2018) and robenidine (Lotz et al. 2024) have been tested. While these efforts to repurpose modified molecules show promise, there is still limited success in finding compounds with higher specificity against schistosomiasis and *in vivo* activity.

In addition to antimalarial drugs, researchers have also explored other sources of compounds in the quest for new anthelmintics that are effective against *Schistosome* spp. infections. Some investigations have focused on screening anticancer drug pipelines due to the large number of compounds marketed for human use (Cowan and Keiser 2015). One promising candidate for repurposing against schistosomiasis is miltefosine, an alkylphospholipid initially developed to treat cutaneous metastases of breast cancer (Eissa et al. 2015). *In vitro* and preclinical studies have shown that miltefosine exhibits significant activity against different developmental stages of *S. mansoni*, either alone or in combination with praziquantel (Eissa et al. 2020). Histone deacetylase inhibitors (HDACi), some of which have been approved for treating various hematological malignancies, have also been identified as potential drug targets for schistosomiasis treatment (Ghazy et al. 2022). However, the results available so far are based mostly on *in vitro* studies, and tests in animal infection models are still required prior to considering HDACi for further preclinical trials as anti-schistosomal candidates. Furthermore, nifuroxazide, an oral nitrofuran antibiotic, has recently been explored to be repurposed for parasitic diseases, including schistosomiasis (Roquini et al. 2023). So far, it has been demonstrated that nifuroxazide has anti-schistosomal properties both *in vitro* and *in vivo*, with significant egg burden reduction in experimentally infected mice.

Repurposable Drugs for the Treatment of Taeniases and Cysticercoses

As mentioned before, the available cestocidal drugs for treating taeniasis and cysticercoses are primarily limited to albendazole, praziquantel, and niclosamide.

However, the extensive use of few drugs poses a risk of parasite resistance. Additionally, these drugs may not be fully effective in treating severe forms of cysticercoses, such as ocular cysticercosis and neurocysticercosis, which further complicates their management (Hamamoto Filho et al. 2022). Despite these challenges, there have been few drugs proposed for repurposing for treating *Taenia* spp. infections in the past four decades. Some of the more recent repurposable alternatives include tribendimidine and tamoxifen.

Tribendimidine is a synthetic anthelminthic drug that was developed in China in the 1980s. It is derived from the p-aminophenylamidine-based anthelmintic amidantel (Xiao et al. 2013). It gained approval from Chinese authorities in the early 2000s for treating human soil-transmitted helminthiases. Subsequently, its potential for repurposing against *Taenia* spp., either alone or in combination with albendazole, was demonstrated in a Phase 2 clinical trial (Steinmann et al. 2008). However, further evaluation is still needed to determine the effectiveness of tribendimidine against taeniasis and cysticercoses. Furthermore, global access to tribendimidine is still limited, and its approval by the U.S. FDA is pending (Hiroshige et al. 2018).

Tamoxifen, an estrogen receptor antagonist, is widely used in the treatment of estradiol-dependent breast cancers. Additionally, it has been suggested as a potential therapeutic drug for various infectious diseases (Montoya and Krysan 2018). The rationale behind the repurposing of tamoxifen against *Taenia* spp. infections stems from the knowledge that steroidal hormones play crucial roles in the biology of these parasites (Escobedo et al. 2010). Tamoxifen has also demonstrated strong cysticidal and anti-taeniasis effects on *T. solium* in hamsters (Escobedo et al. 2013). However, its taenicial and cysticidal potential in human infections remains to be evaluated.

More recent efforts for identifying novel druggable targets and drugs for repurposing against taeniasis and cysticercoses have been facilitated by the whole-genome sequencing of different species of the genus *Taenia*, including *T. solium* (Tsai et al. 2013; S. Wang et al. 2016; Bobes et al. 2022). Some potential *Taenia* spp. druggable targets that have emerged from comparative and functional genomic studies include aminoacyl tRNA synthetases (Goel et al. 2019) and HDACs (Vaca et al. 2019, 2021).

Repurposable Drugs for the Treatment of Echinococcoses

As previously mentioned, current chemotherapy treatments for both alveolar and cystic echinococcoses are based almost exclusively on the oral administration of benzimidazoles, such as albendazole and mebendazole (González-Hernández et al. 2019). However, these benzimidazole drugs only have parasitostatic activity, and to be effective in stopping the growth of metacestodes must be given for long periods (years or even lifetime) and at high doses, which can lead to severe adverse effects that reduce the quality of life of patients and may result in embryotoxicity and teratogenicity (Koziol and Brehm 2015; Lundström-Stadelmann et al. 2019). These issues, coupled with the increasing evidence of resistance or limited effectiveness of benzimidazole drugs in treating medically and veterinary important parasitic helminths (Mortezaei et al. 2019), have prompted research into repurposing drugs for the treatment of *Echinococcus* spp.

Classic studies on drug repurposing against echinococcoses included those made with ivermectin, nitazoxanide, amphotericin B, and antimalarials (Panic et al. 2014). Initial studies carried out with the antinematicidal drug ivermectin showed no effects on *E. multilocularis* larvae in experimental infections in rodents. More recent studies, on the other hand, showed more promising results against *E. granulosus* preadults (protoscolices) *in vitro* by delivering ivermectin using nanostructured lipid carriers (Ahmadpour et al. 2019), but confirmatory *in vivo* studies are still required.

The effects of nitazoxanide, a thiazolide derivative approved for the treatment of intestinal infections caused by *Cryptosporidium* spp. and *Giardia intestinalis*, on *E. multilocularis* and *E. granulosus* metacestodes were evaluated both *in vitro* and *in vivo*, with encouraging outcomes either alone or in combination with albendazole (Stettler et al. 2004; Walker et al. 2004). These anti-echinococcal effects of nitazoxanide have been recently confirmed in multi-assay platforms for *Echinococcus* spp. *in vitro* drug assessment (Kaethner et al. 2023). Initial tests conducted on patients with disseminated cystic echinococcosis demonstrated the potential of nitazoxanide for use in combination therapy with albendazole (Pérez-Molina et al. 2011). However, limitations still exist for treating chronic and extensive body lesions.

For the antifungal agent amphotericin B, data show its inhibitory effects on *E. multilocularis* metacestode growth both *in vitro* and in alveolar echinococcosis patients (Reuter et al. 2010). It has been proposed as a salvage treatment for alveolar echinococcosis in cases of resistance or intolerance to treatment with benzimidazoles (Burkert et al. 2022).

The activity spectrum of certain antimalarial drugs has also been evaluated against echinococcoses. For example, mefloquines and artemisinins have demonstrated effects on both *E. multilocularis* and *E. granulosus* in experimental models, either *in vitro* or *in vivo* (Memedovski et al. 2023; Wen et al. 2023). Mefloquine, combined with amphotericin B, has been proposed as a salvage therapy for alveolar echinococcosis upon the development of albendazole intolerance (Jelicic et al. 2023).

Over the past decade, targeted approaches for repurposing drugs against echinococcoses have greatly benefited from the whole-genome sequencing of different *Echinococcus* species, including *E. multilocularis* and *E. granulosus* (Tsai et al. 2013; Zheng et al. 2013; Maldonado et al. 2017, 2019). The availability of sequence data and subsequent functional analyses have enabled the identification of metabolic vulnerabilities in these parasites, leading to the discovery of potential targets for drug repurposing (Tsai et al. 2013; Cancela et al. 2019). For instance, *Echinococcus* spp. and other parasitic helminths are not capable of synthesizing nucleotides *de novo*; instead, they rely solely on the salvage pathways for nucleotide synthesis. This makes the enzymes associated with these salvage pathways attractive targets for drug repurposing. As evidence of this concept, hydroxyurea, an anticancer drug that inhibits ribonucleotide reductase (a component of nucleotide salvage pathways), has been shown to inhibit DNA synthesis and effectively kill *E. granulosus* protoscoleces *in vitro* (Panesso et al. 2023).

Repurposable Drugs for the Treatment of Foodborne Trematodiases

Drug repurposing efforts have also been undertaken to treat various foodborne

trematodiases caused by liver or lung flukes belonging to the genera *Clonorchis*, *Opisthorchis*, *Fasciola*, and *Paragonimus*. As happens for other main helminthiases, such efforts are warranted due to the limited availability of approved drugs, which, in the case of foodborne trematodiases are primarily praziquantel, triclabendazole, niclosamide, and albendazole.

The drug sources for repurposing against foodborne trematodiases have ranged from expanding applications of broad-spectrum anthelmintics to anti-protozoal drugs. Among repurposable anthelmintics, oxfendazole, a benzimidazole used in the United States and Europe against roundworms and tapeworm infections in ruminants, has been assessed for the treatment of human fluke infections (Pfarr et al. 2023). Tribendimidine, initially developed and used as an anthelminthic drug against soil-transmitted worm infections, has also been under assessment for repurposing against flukes, including *C. sinensis* and *O. viverrini* (Qian et al. 2022).

The effectiveness of antiprotozoals against foodborne trematodiases is well illustrated by the cases of nitazoxanide, artemisinins, and mefloquines. Nitazoxanide has been evaluated in various clinical trials conducted in different countries as an alternative treatment for *F. hepatica* infections, particularly in cases where triclabendazole treatment has been unsuccessful (Ramadan et al. 2019). Artemisinines and mefloquine, currently being evaluated for repurposing against schistosomiases, have shown promising effects on at least some species of food-borne trematodes in rodent *in vivo* studies. Artemisinins have been shown to be very effective against the liver flukes *F. hepatica* and *C. sinensis*, but less effective against *O. viverrini* (Panic et al. 2014). Mefloquine, on the other hand, has shown no activity against *F. hepatica* and *C. sinensis*, but has shown effects against *O. viverrini* and *Paragonimus westermani* (Panic et al. 2014). Studies with artemisinins and mefloquine, either alone or in combination, have progressed to human trials with patients infected by *Fasciola* spp., *C. sinensis*, or *O. viverrini*, but still with limited success (Lam et al. 2018).

In recent years, quinazoline derivatives, some of which have been approved for human use as antibiotics or anticancer drugs, have also become a source for repositioning as flukicides. Phenotypic and computational screenings and targeted-based approaches have identified quinazoline-derived compounds with flukicide action (Botros et al. 2019; Sebastian-Perez et al. 2020; Sprague et al. 2024). For instance, benzamidoquinazolinone analogs targeting $TRPM_{PZQ}$ (the schistosome ion channel targeted by praziquantel) presented broader flukicide spectra than praziquantel itself, with at least one of them (called BZQ) being potentially effective for *F. hepatica* and all other profiled flukes, including *F. gigantica*, *C. sinensis*, and *O. viverrini* (Sprague et al. 2024).

Concluding Remarks

Helminthiases are neglected diseases that primarily affect developing countries, resulting in significant medical, social, and economic burdens on vulnerable populations. The effective treatment, prevention, control, and potential eradication of these diseases depends, in part, on the availability of reliable anthelmintic drugs. However, the current arsenal of anthelmintic drugs is limited, with few options

that have limitations in terms of efficacy and spectrum of action. Furthermore, the emergence of drug resistance poses a risk to these existing drugs.

There is an urgent need for the development of novel and more efficient anthelmintic drugs to strengthen the available arsenal. Unfortunately, the high cost associated with drug discovery and development has resulted in a lack of interest from the global pharmaceutical industry in investing in anthelmintic drug research. In this context, drug repurposing offers a solution by identifying potential anthelmintic effects among compounds already approved for treating other human illnesses. Some broad-spectrum anthelmintics intended initially for veterinary use have also been explored as alternative treatment options for human helminthiases. Additionally, certain antibiotics, antiprotozoals, and anticancer agents have shown considerable efficacy as anthelmintic agents.

Various strategies for drug repositioning show promise in discovering novel anthelmintic agents, leveraging existing drugs and molecular knowledge to address the global burden of parasitic worm infections. By combining computational, experimental, and systems-based approaches, researchers can identify repurposed drugs with diverse mechanisms of action, optimize treatment regimens, and expedite the translation of preclinical discoveries into clinical applications. Continued innovation and collaboration in drug repositioning research can revolutionize anthelmintic drug discovery and advance efforts to control and eliminate helminth diseases worldwide.

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CHAPTER

8

Applications of Nanomaterials for Drug Repurposing

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Introduction

Nanomaterials, particularly nanoparticles (NPs), have revolutionized drug design and delivery, driving significant progress in medical treatments (Sousa Ribeiro et al. 2021; Abdel-Mageed et al. 2021; Namiot et al. 2023). Their unique attributes—including their size, diverse composition, ability to incorporate both hydrophilic and lipophilic drugs, high surface area-to-volume ratio, and potential for functionalization with specific ligands—help to minimize side effects and maximize therapeutic efficacy (Czech et al. 2019; Lotierzo et al. 2020; Sousa Ribeiro et al. 2021; Ahmad et al. 2021). This has led to several nanomedicines receiving the Food and Drug Administration (FDA) approval, marking a significant milestone in integrating nanotechnology into clinical practice (Barenholz 2012; Ma and Mumper 2013; Silverman and Deitcher 2013; Milano et al. 2022).

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In drug design, nanomaterials offer unmatched advantages. They improve drug delivery, alter biodistribution and pharmacokinetic properties, enable controlled and sustained release, facilitate targeted delivery, and support combination therapies by incorporating multiple drugs (Czech et al. 2019; Lotierzo et al. 2020; Sousa Ribeiro et al. 2021; Ahmad et al. 2021). This level of precision enhances therapeutic outcomes and reduces systemic toxicity, paving the way for more effective and personalized treatments (Sousa Ribeiro et al. 2021).

FDA has acknowledged the potential of nanomedicines by approving various products that showcase the practical benefits of nanotechnology in healthcare (Barenholz 2012; Ma and Mumper 2013; Silverman and Deitcher 2013; Milano et al. 2022). For instance, Doxil®, a liposomal formulation of doxorubicin, has been a breakthrough in treating cancers with reduced cardiac toxicity (Barenholz 2012). Abraxane®, which features paclitaxel in albumin NPs, effectively treats various cancers while avoiding problematic solvents (Ma and Mumper 2013). Marqibo® highlights the advantages of liposomal formulations for treating blood cancers (Silverman and Deitcher 2013), and Onivyde® demonstrates improved delivery of irinotecan for pancreatic cancer (Milano et al. 2022).

These FDA-approved nanomedicines exemplify how nanotechnology addresses complex challenges in drug delivery and therapeutic efficacy. They represent a shift towards more targeted, effective, and safer treatments, underscoring the transformative potential of nanomaterials in modern medicine.

Drug Repurposing and Nanomaterials

Developing new drugs is complex, time-consuming, and expensive, taking 10–15 years and costing $1–2 billion to reach clinical use approval (Pushpakom et al. 2018; Xia et al. 2024). This lengthy timeline and expense are driven by mandatory preclinical research and subsequent human clinical trials (Figure 1). Preclinical studies use human cells and animal models to assess drug toxicity, efficacy, pharmacokinetics, and pharmacodynamics. Positive outcomes lead to Phase I, II, and III trials in humans to confirm safety and efficacy (Pushpakom et al. 2018; Xia et al. 2024).

Considering these various stages involved in new drug development, drug repurposing has emerged as a strategy to reduce time and costs, thereby expediting access to innovative treatments for patients (Pushpakom et al. 2018; Xia et al. 2024). Drug repurposing, or drug repositioning, involves exploring new therapeutic uses for approved or investigational drugs beyond their original medical indication (Pushpakom et al. 2018; Kulkarni et al. 2023; Xia et al. 2024). This practice can be divided into two categories: “Hard” and “Soft” repurposing (Pantziarka et al. 2018; WHO 2021). “Hard” repurposing means using a drug for a different medical purpose, like using antibiotics to treat cancer. “Soft” repurposing involves using a known medication within the same medical area, such as using established cancer drugs for new types of cancer (Pantziarka et al. 2018; WHO 2021). Despite the need for rigorous clinical trials, repurposed drugs carry a significantly lower risk of failure compared to developing entirely new drugs since they have already demonstrated safety in preclinical studies and human trials (Pushpakom et al. 2018; Yang et al.

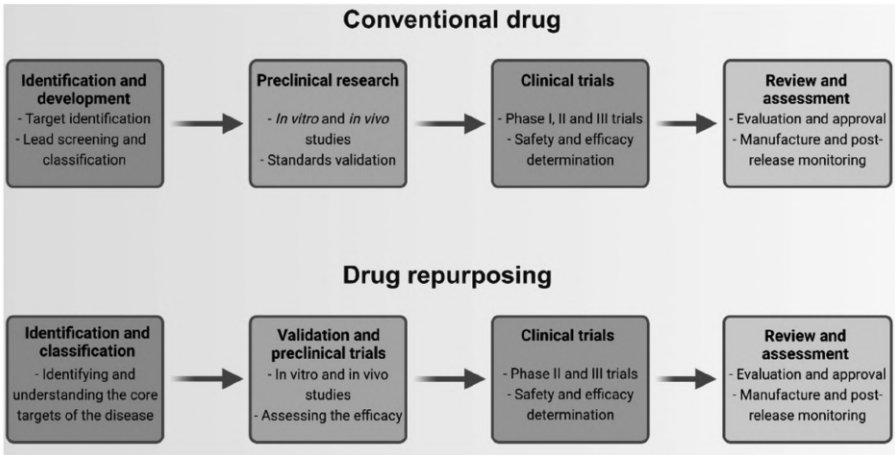
Conventional drug
Identification and development
- Target identification
- Lead screening and classification
Preclinical research
- *In vitro* and *in vivo* studies
- Standards validation
Clinical trials
- Phase I, II and III trials
- Safety and efficacy determination
Review and assessment
- Evaluation and approval
- Manufacture and post-release monitoring
Drug repurposing
Identification and classification
- Identifying and understanding the core targets of the disease
Validation and preclinical trials
- In vitro and in vivo studies
- Assessing the efficacy
Clinical trials
- Phase II and III trials
- Safety and efficacy determination
Review and assessment
- Evaluation and approval
- Manufacture and post-release monitoring

Figure 1: Traditional drug discovery versus drug repurposing process and development. ⏎

2020; Kulkarni et al. 2023; Xia et al. 2024). Thalidomide provides a clear example of the success of drug repurposing (Pushpakom et al. 2018; Xia et al. 2024). Initially used as a sedative, it was later found to cause severe congenital disabilities in pregnant women. However, it was subsequently discovered to be effective in treating leprosy and multiple myeloma, leading to sales of $8.2 billion in 2017 (Pushpakom et al. 2018; Xia et al. 2024).

Despite the promising approach of drug repurposing, numerous medicines currently available are hindered in their therapeutic effectiveness by physical and chemical properties (Czech et al. 2019; Mitchell et al. 2021; Usharani et al. 2024). Issues like poor water solubility, limited stability, incompatibility with administration routes, and insufficient cellular uptake lead to ineffective treatments and often undesirable side effects, posing significant concerns for human health (Czech et al. 2019; Mitchell et al. 2021; Usharani et al. 2024). Recognizing these challenges, researchers have identified nanotechnology as a promising solution (Figure 2) (Czech et al. 2019; Mitchell et al. 2021; Usharani et al. 2024). In drug repurposing, the unique properties of NPs, such as their ability to enhance drug solubility, provide targeted delivery, and offer controlled release, are crucial, as these features enable existing medications to be more effective and reach their intended therapeutic targets with greater precision (Czech et al. 2019; Lotierzo et al. 2020; Sousa Ribeiro et al. 2021; Ahmad et al. 2021). A notable example of this is the use of nanomaterials to improve Alzheimer's disease treatments. Studies indicate that more than 98% of small-molecule drugs and almost 100% of biological drugs cannot cross the blood-brain barrier (BBB) effectively, which hampers their ability to treat Alzheimer's disease. However, this challenge might be addressed by using NPs (Roghani et al. 2024).

Numerous studies involve NPs and drug repurposing, and this number grows year after year (Figure 3). These studies focus on diseases such as cancer (Yurtdaş-Kırımlıoğlu et al. 2016; Garg and Garg 2018; Liu et al. 2019; Vaidya et al. 2020),

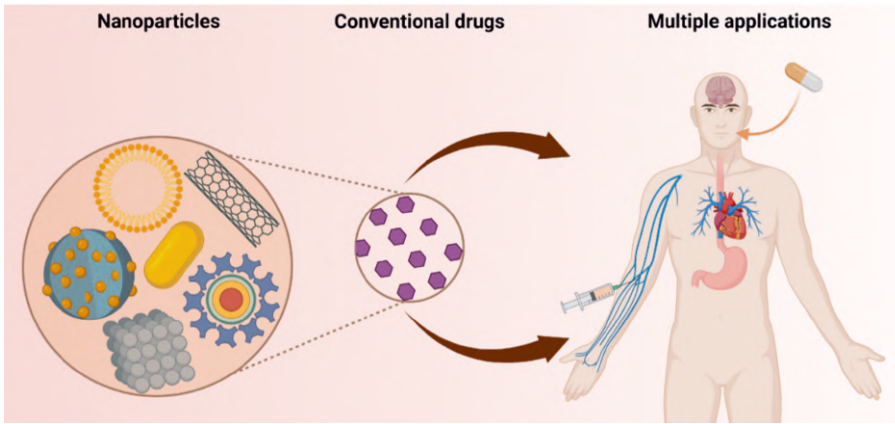

Figure 2: Diagram illustrating the integration of drug repurposing with nanomaterials. ⏎

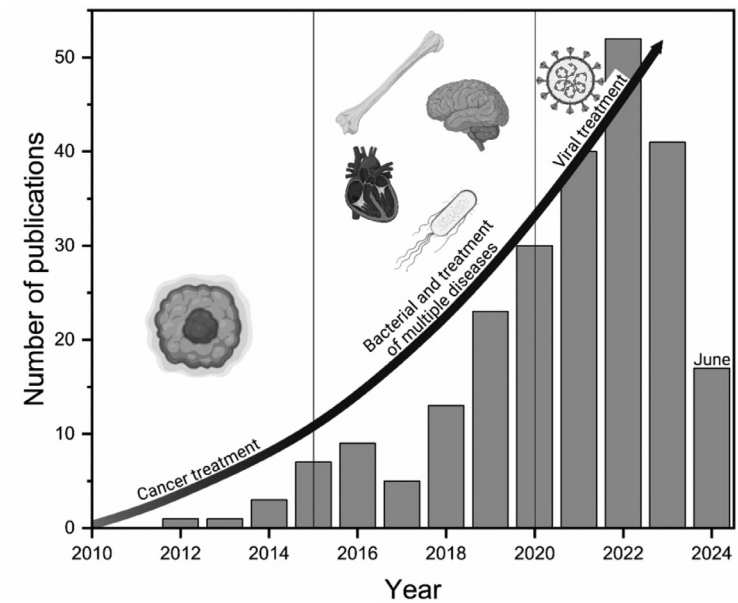

Figure 3: Number of publications referencing "drug repurposing" and "nanoparticle" year-on-year over the last 14 years. (Statistics from the Web of Science database.) ⏎

neurodegenerative diseases (Alzheimer's and Parkinson's disease) (Muntimadugu et al. 2016; González-Lizárraga et al. 2017; Lopalco et al. 2018; Jojo et al. 2019; Zhao et al. 2020), viral infections (Joshy et al. 2018; Garg et al. 2019; Sanna et al. 2022), heart diseases (Ikeda et al. 2016; Giannouli et al. 2018; Boarescu et al. 2019; Mosa et al. 2021; Gendron et al. 2021; Li et al. 2022), and leishmaniasis (Da costa-Silva et al. 2017; Mehravaran et al. 2019; Romanelli et al. 2019; Santamaría-Aguirre et al. 2024), which will be discussed in more detail in the next section.

Applications of Nanomaterials and Drug Repurposing for Disease Treatment

Cancer

Cancer is one of the leading diseases of our time, causing approximately 9.6 million deaths worldwide in 2018 (Yang et al. 2020). Conventional treatments, such as chemotherapy and radiotherapy, are not selective and require high doses of drugs, which often lead to serious side effects. As previously mentioned, the association of NPs with drug repurposing is a promising alternative for enhancing treatment efficiency and reducing the time and cost required for developing new drugs (Yang et al. 2020). Currently, the literature comprises a series of studies on nanomaterials applied to drug repurposing, aiming at cancer treatment (Garg and Garg 2018; Liu et al. 2019; Yang et al. 2020; Yang and Shi 2020). For instance, Liu et al. incorporated aspirin as one of the constituents in a nanocomplex designed for breast cancer therapy. Aspirin (acetylsalicylic acid) is commonly prescribed for alleviating fever, headaches, muscle pains, and other conditions. In their research, the authors devised a system for co-loading the chemotherapeutic agent docetaxel and aspirin within micelles. Their findings revealed that heparanase, overexpressed in cancer cells, can degrade the heparan sulfate of micelles, thereby facilitating the release of aspirin and docetaxel, thus initiating a cancer-specific therapeutic response. The study demonstrated that aspirin played a significant role in inhibiting tumor growth compared to nanocomplexes lacking aspirin. Originally employed in treating infections stemming from wounds and burns, curcumin has also been linked with nanomaterials and demonstrated efficacy against cancer (Garg and Garg 2018; Yang et al. 2020). Garg and Garg integrated curcumin into silver NPs and observed that it induced cytotoxicity in tumor cells in a dose-dependent manner and yielded a more potent antitumor effect compared to plain curcumin. Ibuprofen, a non-steroidal anti-inflammatory, is another drug that has been incorporated into nanomaterials for cancer treatment (Yurtdaş-Kırımlıoğlu et al. 2020; Xia et al. 2024). Yurtdaş-Kırımlıoğlu et al. described a study incorporating this drug into EudragitVR RS 100 and/or octadecylamine-modified poly-lactic/glycolic acid (PLGA) NPs for lung cancer treatment. These NPs showed a highly specific affinity for human lung adenocarcinoma cells, and the effect of ibuprofen was increased by 4–28 times compared to its pure form. Like ibuprofen, quinacrine has been combined with nanomaterials for treating lung cancer (Vaidya et al. 2020). Vaidya et al. integrated quinacrine, originally an anti-malarial drug, into positively charged inhalable PLGA NPs, employing polyethyleneimine (PEI) as a stabilizer and bovine serum albumin (BSA) as a charge-neutralizing coating material. They noted that PEI's use enhanced the entrapment efficiency of quinacrine in NPs, while BSA improved the release profile and cellular uptake. Studies on anti-proliferative activity demonstrated the enhanced therapeutic efficacy of quinacrine-loaded NPs over plain quinacrine.

Neurodegenerative Diseases

Alzheimer's and Parkinson's rank among the most prevalent neurodegenerative conditions globally. Addressing these diseases presents significant hurdles, primarily

due to the brain's restricted accessibility (Séguy et al. 2022; Hernandéz-Parra et al. 2022). Conventional medications employed for treating such conditions often have limited efficacy and can potentially result in severe side effects (Séguy et al. 2022; Hernandéz-Parra et al. 2022). To address these challenges, leveraging both nanomaterials and drug repurposing has emerged as a promising approach to enhance the treatment of neurodegenerative diseases (Séguy et al. 2022; Hernandéz-Parra et al. 2022). For instance, Tarenflurbil, an enantiomer of the anti-inflammatory medication flurbiprofen, exhibited promise in treating Alzheimer's during Phase II clinical trials. However, its efficacy faltered in Phase III primarily due to inadequate penetration of the BBB (Séguy et al. 2022). To overcome this challenge, Muntimadugu et al. (2016) encapsulated the drug in both PLGA polymeric and solid lipid NPs. These formulations demonstrated favorable distribution within the brain, as highlighted by the authors. Moreover, Tarenflurbil's overall pharmacokinetic performance was enhanced when administered via NPs compared to traditional solution or suspension forms. Pioglitazone, a medication commonly prescribed for Type II diabetes, encountered a similar challenge. Despite showing promise in preclinical studies as a treatment for Alzheimer's, its limited ability to penetrate the blood-brain barrier and its severe peripheral side effects posed obstacles during clinical trials (Séguy et al. 2022). In response, Jojo et al. devised a solution by formulating pioglitazone into a lipid-based nanoformulation. This formulation, composed of a blend of Tween 80 and Pluronic F68 and supplemented with a positive charge inducer, stearyl amine, aimed to improve mucoadhesion. The positive charge facilitated interaction with negatively charged sialic acid groups on the mucosal surface, thus reducing mucociliary drainage. Using this formulation, the authors demonstrated a significant enhancement in pioglitazone's permeability across the nasal mucosa *ex vivo*. Safety assessments confirmed the formulation's suitability for *in vivo* administration. Furthermore, direct nose-to-brain transport substantially increased the concentration of pioglitazone reaching the brain *in vivo*.

In the context of Parkinson's disease, nanomaterials have also been instrumental in significantly enhancing the effectiveness of existing medications (Hernandéz-Parra et al. 2022). It is known that the absence of dopamine is recognized as a catalyst for basal ganglia dysfunction, the underlying physiological mechanism of Parkinson's disease (Hernandéz-Parra et al. 2022). Hence, utilizing this molecule as a medication holds allure. Nevertheless, like previously discussed medications, dopamine faces challenges traversing the BBB, limiting its efficacy (Hernandéz-Parra et al. 2022). Based on this understanding, incorporating dopamine into NPs has become a promising avenue (Lopalco et al. 2019; Hernandéz-Parra et al. 2022). Lopalco et al. (2019) encapsulated the hydrophilic drug dopamine hydrochloride within transferrin-functionalized and unfunctionalized liposomes. Their findings indicate that the apparent permeability is increased with transferrin-functionalized nanocarriers, making this system a safe and efficient option for drug delivery to the brain. Ginkgolide B (GB), derived from Ginkgo biloba leaves, is also frequently investigated as a neuroprotective treatment for Parkinson's. However, its poor water solubility results in minimal exposure in the brain and bloodstream, limiting its clinical usefulness (Zhao et al. 2020; Hernandéz-Parra et al. 2022). To address

this challenge, Zhao et al. utilized FDA-approved polymers Poly(ethylene glycol) (PEG) and poly(ε-caprolactone) (PCL) to create NPs for encapsulating GB. Their research illustrated that these NPs effectively encapsulate significant quantities of GB and notably enhance its accumulation in the brain. Furthermore, González-Lizarraga et al. (2017) investigated the repurposing of the antibiotic doxycycline as a therapeutic agent for synucleinopathies by modulating α-synuclein oligomerization. Synucleinopathies are neurodegenerative disorders marked by the abnormal buildup of α-synuclein protein aggregates in neurons, nerve fibers, or glial cells. Their study demonstrated that doxycycline alters the structural conformation of α-synuclein oligomers, promoting the formation of non-toxic parallel β-sheet structures while reducing cytotoxicity. The authors used a series of assays, like lactate dehydrogenase release and calcein leakage, to evidence the different effects of on-pathway (absence of doxycycline) and off-pathway (presence of doxycycline) oligomers on biomimetic membrane integrity. Their findings suggest that doxycycline may offer a novel strategy for neuroprotection in synucleinopathies by mitigating the harmful effects of α-synuclein aggregation, warranting further investigation into its therapeutic potential.

Viral Infections

Recently, we have witnessed the coronavirus disease 2019 (COVID-19) emerge as a significant threat to global public health. According to the World Health Organization (WHO), it is caused by the novel coronavirus severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and has resulted in the deaths of nearly 15 million people worldwide (Ahmad et al. 2021). Most therapeutic compounds originally intended for another disease were assessed for their potential effectiveness against COVID-19 (repurposing) (Ahmad et al. 2021). However, utilizing existing antivirals presents numerous challenges, including inadequate cellular uptake, limited solubility and availability in water, reduced biological potency, a disproportionate risk-to-benefit ratio, and increased toxicity, all of which impede their efficacy (Ahmad et al. 2021; Delshadi et al. 2021). In order to overcome these obstacles, various nanomedicine-based platforms have emerged to enhance the therapeutic potential of repurposed antiviral medications (Ahmad et al. 2021; Delshadi et al. 2021; Yasamineh et al. 2022). For instance, remdesivir, which was initially developed as an antiviral for Ebola virus treatment, received emergency use authorization from the FDA for treating hospitalized COVID-19 patients with severe symptoms. However, its effectiveness was hampered by its inadequate physicochemical and pharmacokinetic properties (Sanna et al. 2022). In response, Sanna et al. devised drug-encapsulated polymeric NPs densely decorated with various ligands to selectively target ACE2 enzyme receptors. They discovered that these modified NPs are more efficiently bound to ACE2 receptors than non-targeted ones, resulting in a significantly enhanced antiviral effect compared to the free drug at equivalent concentrations. Currently, concerning SARS-CoV-2, our reliance is on vaccines. However, it is important to emphasize that SARS-CoV-2 is just one of several viruses. Therefore, increasing the effectiveness of existing antivirals becomes crucial for proactive preparation against pandemics, reducing infection symptoms, and preserving lives.

For instance, zidovudine is a widely utilized antiviral medication in combating HIV/AIDS; however, its effectiveness is limited (Joshy et al. 2018; Delshadi et al. 2021). In response, Joshy et al. (2018) introduced a new nanosystem comprising dextran, lipid, and PEG to encapsulate this drug. The lipid and PEG components endowed the nanosystems with stability and facilitated controlled drug release. Furthermore, they observed a notable enhancement in the efficiency of brain cellular internalization of the hybrid NPs. As per the authors, these findings underscore the potential and effectiveness of hybrid NPs for delivering zidovudine efficiently. Another example involves lopinavir, employed in the treatment of HIV-associated neurocognitive disorder (Garg et al. 2019; Ahmad et al. 2021). Despite its potential, this drug demonstrates inadequate oral bioavailability and distribution within the brain (Garg et al. 2019; Ahmad et al. 2021). Acknowledging this limitation, Garg et al. devised nanolipidic carriers for lopinavir to target the brain, aiming to mitigate HIV-associated neurocognitive disorder. The study reported that the formulation containing compritol, oleic acid, and Tween 80 enhanced the systemic availability of the drug, leading to notably higher drug concentrations in the brain compared to the pure drug suspension. While these two cases are not direct examples of drug repurposing, they illustrate how nanomaterials can improve the efficiency of drugs already on the market.

Cardiovascular Diseases

Cardiovascular diseases constitute the leading cause of death globally, with projections estimating 23.6 million deaths by 2030 (Yusuf et al. 2020; Yang et al. 2022). These diseases encompass a group of conditions involving the heart, arteries, veins, and blood itself (Tripathi et al. 2024). Among these conditions are arrhythmias, heart failure, deep vein thrombosis, atherosclerosis, myocardial infarction, coronary heart disease, peripheral arterial disease, inflammatory heart disease, and other related disorders (Yang et al. 2022). Treatment for cardiovascular diseases often involves using medications, which are frequently combined (Chopra et al. 2022). However, the long-term prognosis of these medications is still poor, reducing patient adherence and leading to unpredictable consequences (Guo et al. 2023; Onódi et al. 2023). Therefore, there is an urgent demand for new therapeutic strategies.

Giannouli et al. (2018) conducted a study aimed at preventing cardiovascular diseases by incorporating quercetin into PLGA NPs. Quercetin is widely known for its valuable antioxidant, neurological, antiviral, and anticancer properties; however, its use is limited due to its poor water solubility and instability in physiological mediums. The study revealed that embedding quercetin in NPs protected the drug against degradation and facilitated controlled release, essential factors for improving its efficacy against cardiovascular diseases. Curcumin is another antioxidant that has been investigated for its cardioprotective effects; nevertheless, its rapid systemic clearance and low solubility restrict its clinical application. To address this challenge, Boarescu et al. (2019) encapsulated curcumin in polymer-based NPs and evaluated its effects compared to pure curcumin. They found that these curcumin NPs significantly enhance cardioprotection by exerting antioxidative and anti-inflammatory effects in cases of acute myocardial infarction in diabetes mellitus.

Curcumin NPs were also studied by Mosa et al. (2021), and this time, they were combined with chitosan nanoformulations. The authors found that combining the nanoformulations of both antioxidants had beneficial effects in mitigating the inflammatory response after cardiac infarction induced by hydroxyapatite NPs. Cyclosporine, recognized for its immunosuppressive properties, is also utilized for cardioprotection (Gendron et al. 2021). However, its distribution and physicochemical properties restrict intravenous administration (Gendron et al. 2021). To address this challenge, Gendron et al. created a nanoformulation by covalently conjugating squalene (a biodegradable lipid) to cyclosporin A. Conjugating therapeutic molecules to squalene has been demonstrated to extend blood circulation time, offer compelling targeting properties, and reduce toxicity. The study demonstrated a potent cardioprotective effect in cardiomyoblasts exposed to experimental hypoxia/reoxygenation. Cyclosporine's role as a cardioprotector was also investigated using PLGA and biomimetic NPs synthesized from 5,5-dimethyl-4,6-dithio-propylene glycol azelate. In both studies, NPs containing cyclosporine showed enhanced cardioprotective effects (Ikeda et al. 2016; Li et al. 2022).

Leishmaniasis

Leishmaniasis is an infectious parasitic disease caused by protozoans of the genus Leishmania. The disease is transmitted through vectors such as Phlebotomus and Lutzomyia sandflies, insects capable of transmitting the parasite to various mammals (Torres-Santos 2018). This disease is classified as one of the 20 neglected tropical diseases by the WHO, with its cutaneous form being the most common clinical presentation (Reithinger et al. 2007; WHO 2010; Hartley et al. 2013; El-Dirany et al. 2022). Based on certain studies, human actions such as deforestation, global migration, climate change, urbanization, immigration to endemic areas, and military operations have made this disease more likely worldwide (Rodriguez-Morales et al. 2009; Kevric et al. 2015).

There are two main lines of treatment for this disease (Burza et al. 2018; Casadei et al. 2021; de Almeida et al. 2024). The first line uses complexes of the pentavalent antimony ion, namely meglumine antimoniate and sodium stibogluconate. However, due to the emergence of resistant species, there has been a decline in the effectiveness of these drugs (Burza et al. 2018; Casadei et al. 2021; de Almeida et al. 2024). Another treatment line has emerged, involving the use of medications such as pentamidine, paromomycin, miltefosine, and amphotericin B, which are administered parenterally. Nevertheless, some of these available drugs with anti-leishmanial activity are limited in use due to their high toxicity and resistance (Burza et al. 2018; Casadei et al. 2021; de Almeida et al. 2024). Therefore, seeking new low-cost treatments and strategies is essential. In order to achieve this goal, several research groups worldwide are employing the drug repurposing approach combined with the use of nanomaterials (Casadei et al. 2021). For example, delamanid, initially developed for multidrug-resistant tuberculosis, has demonstrated efficacy against the intracellular amastigote form of the parasite, suggesting potential as a therapeutic option for the disease (Antamaría-Aguirre et al. 2024). However, improvements are required to enhance its bioavailability, ensure sustained release for prolonged therapeutic effects, and

improve chemical stability. Santamaría-Aguirre et al. (2024) encapsulated delamanid in solid lipid NPs to address these challenges. Their study showed that this formulation enabled efficient skin penetration upon topical application and enhanced activity against intracellular amastigotes. Another study combining drug repurposing with nanomaterials was conducted by Mehravaran et al. (2019). In their research, the authors utilized imiquimod, a topical chemotherapy agent used for treating skin tumors, and incorporated it into cationic liposomes. It is worth noting that cationic liposomes are renowned for their clinical safety profile, low immunogenicity, depot effects, and ease of preparation (Quer et al. 2012; Mehravan et al. 2019). According to the authors (Mehravaran et al. 2019), this formulation induced a cellular immune response against the parasite responsible for the disease, underscoring its potential in treating leishmaniasis. Sertraline, typically prescribed for depression, anxiety disorders, post-traumatic stress disorder, obsessive-compulsive symptoms, and premenstrual dysphoric disorder, is also under evaluation for managing leishmaniasis (Casadei et al. 2021). However, in its unencapsulated form, it shows limited oral absorption and therefore low efficacy (Garza-Tovar et al. 2020; Casadei et al. 2021). Romanelli et al. (2019) developed phosphatidylserine liposomes containing sertraline, showing that this formulation effectively reduced parasite levels at low doses and targeted delivery to the liver and spleen of infected mice. Some veterinary drugs have also shown promise against leishmaniasis. For instance, Costa-Silva et al. (2017) combined a negatively charged nanoliposome with buparvaquone, a drug used in veterinary medicine to treat bovine theileriosis. They showed that this combination has immunomodulatory effects on host cells, effectively eliminating the parasite at low doses in animal models.

Perspectives

Despite its promise, drug repurposing faces significant challenges due to limited investment (Cha et al. 2018; Albuquerque et al. 2022; Mørch 2023). Drugs not covered by regulatory or basic patent protections lack financial incentives, which hinders thorough research (Cha et al. 2018; Albuquerque et al. 2022; Mørch 2023). Currently, three primary groups are studying the drug repurposing method: academia and research institutes, the pharmaceutical industry, and technology companies (Cha et al. 2018). Each group operates with distinct incentives and expectations; for instance, academia is less driven by commercial success pressures than the pharmaceutical industry (Cha et al. 2018). Consequently, several initiatives have emerged to support academic institutions in preliminary studies on established drugs for new indications (Cha et al. 2018; Albuquerque et al. 2022; Mørch 2023). Positive results from these studies may lead to partnerships among these groups, increasing the likelihood of successfully introducing existing molecules to new uses (Cha et al. 2018; Albuquerque et al. 2022; Mørch 2023). Moreover, these initiatives aim to facilitate the integration of emerging fields like nanotechnology into drug repurposing efforts, enabling the development of more efficient systems (Cha et al. 2018; Mørch 2023). This chapter highlights the advantages of NPs in drug studies, including controlled drug delivery, modification of biodistribution and pharmacokinetic properties, targeted therapy, and support for combination treatments with multiple drugs. Considering these

advantages and fostering appropriate partnerships, the fusion of drug repurposing and nanotechnology emerges as a promising platform to swiftly and affordably develop new treatment modalities that effectively meet the diverse needs of patients with various diseases. It is important to emphasize that although existing FDA-approved nanomedicine-based products on the market may not strictly adhere to the traditional definition of "drug repurposing," they demonstrate the potential of nanomedicine to enhance therapeutic outcomes for established medications. As mentioned earlier, notable examples of approved nanomedicines that improve the delivery and effectiveness of existing drugs include Doxil (Barenholz 2012) and Abraxane (Ma and Mumper 2013), among others that specifically target cancer treatment.

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CHAPTER

9

A Review of the Use of Existing Vaccines as a First Line of Defense in the SARS-CoV-2 Pandemic

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Introduction

When the SARS-CoV-2 outbreak emerged, our immune system was naive to the new virus, which caused significant mortality. Most governments considered it necessary to impose severe lockdowns of varying degrees globally to curb the spread of the virus. Scientists began a race against time to develop an effective vaccine. However, this process took several months. In December 2019, the first cases of an unidentified respiratory disease were reported in Wuhan, China (Mohan and Nambiar 2020). On January 9, 2020, the World Health Organization (WHO) announced that a new coronavirus (later named SARS-CoV-2) was the cause of the pneumonia cases and declared that the disease had reached pandemic status (Cucinotta and Vanelli 2020). In the spring of 2020, clinical trials of vaccines against SARS-COV-2 began in humans. Pharmaceutical companies and researchers worldwide accelerated the

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development of vaccines using different technologies, such as those based on mRNA, viral vectors, and recombinant proteins (Krammer 2020).

In November of that year, Pfizer announced that its mRNA vaccine had demonstrated 95% efficacy in Phase 3 clinical trials (Polack et al. 2020), while Moderna reported that its mRNA vaccine was 94.5% effective (Baden et al. 2021). The United Kingdom was the first country to approve the Pfizer vaccine against COVID-19, with the first doses being administered on December 8, 2020 (Mahase 2020). On December 11 and 18, the United States Food and Drug Administration (FDA) approved emergency utilization of the Pfizer and Moderna vaccines, respectively (Fortner and Schumacher 2021). In summary, nearly a year elapsed from the appearance of the first COVID-19 cases in December 2019 to the administration of the first vaccines in December 2020. This rapid development and logistical deployment were an unprecedented achievement and represented an extraordinary collaboration between scientists, governments, and pharmaceutical companies.

By the end of 2020, 1,813,188 COVID-19 deaths were reported to the WHO. However, according to most recent calculations, there were at least 3,000,000 deaths worldwide in 2020 that can be attributed to the COVID-19 pandemic—1.2 million more deaths than officially documented (WHO 2021). More than 3,000,000 deaths in 1 year is a very high number, so this chapter will analyze whether the proposal to use pre-existing vaccines represents a viable option to be used as a first line of defense against pandemic viruses while a specific vaccine is developed. The concept, benefits, and challenges of vaccine repurposing will be described below.

The Concept and Immune Mechanisms Behind Vaccine Repurposing

Vaccine repurposing consists of the utilization of currently available vaccines to fight diseases other than those for which they were created to protect (Saha et al. 2020; Chumakov et al. 2020, 2021; Sultana et al. 2020). By utilizing the well-established safety profiles, manufacturing procedures, and distribution systems of current vaccines, this approach offers a potentially quicker and more economical option than developing new vaccines from scratch (Rathod et al. 2021). Vaccines have historically been considered one of the most effective public health strategies, and repurposing them could expand their benefits to a wider spectrum of illnesses (Saha et al. 2020).

Immune System Modulation Differs Between Live and Non-live Vaccines

The dominant paradigm in vaccinology mostly emphasizes adaptive immunity, which is based on T cells and antibodies specific to certain pathogens. However, several advantageous non-specific effects (NSEs) also contribute to the impact of vaccines (Benn et al. 2020). Comprehensive research has shown that vaccines can have beneficial or harmful NSEs, challenging the long-held view that their effects are limited to the disease they were designed for (Benn et al. 2020). Table 1 shows the most common live and non-live vaccines currently applied.

Table 1. Examples of live and non-live vaccines commonly used in vaccination protocols ⏎

Type of Vaccine	Examples
Live Vaccines	Measles, Mumps, Rubella (MMR) Varicella (Chickenpox) Oral Polio Vaccine (OPV) Bacillus Calmette-Guérin (BCG) Rotavirus Smallpox (Vaccinia)
Non-live Vaccines	Diphtheria, tetanus, and pertussis (DTaP) Inactivated Polio Vaccine (IPV) Hepatitis A and B Influenza (injectable) Human papillomavirus (HPV) Pneumococcal Meningococcal Haemophilus influenza type b (Hib)

The concept of NSEs explains that certain non-live vaccines (e.g. DTaP) are linked to higher all-cause mortality in girls (Aaby et al. 2016b), while all live vaccines substantially decrease all-cause mortality in vaccinated communities compared to unvaccinated ones, more than can be attributed solely to protection against the target pathogen (Kristensen et al. 2000; Aaby et al. 2003; Benn et al. 2013, 2020; Aaby et al. 2016b; Aaby and Benn 2019). For a comprehensive review see (Rubio-Casillas et al. 2024). Additional proof of the off-target protective benefits of live vaccines comes from animal experiments. For instance, mice immunized with the live-attenuated influenza H3N2 vaccine were protected against the respiratory syncytial virus (RSV) challenge, which otherwise resulted in leukocyte infiltration of the respiratory tract and cytokines production (Lee et al. 2018). As a control, immunization with the inactivated influenza vaccine did not protect against RSV infection. Mice lacking Toll-like receptors (TLR3/TLR7) had a lower response to the attenuated influenza vaccine, which is consistent with other data linking these pathways to the activation of innate immunity (Lee et al. 2018). A whooping cough experimental vaccine derived from live-attenuated *Bordetella pertussis* protected mice against inflammation from unrelated respiratory illnesses, including influenza and RSV (Cauchi and Locht 2018).

The Bacillus Calmette-Guérin (BCG) Vaccine

The live attenuated tuberculosis (TB) vaccine, also named BCG, is among one of the most widely used vaccines, having been inoculated more than 4,000,000,000 times worldwide, with an additional 100,000,000 BCG vaccines for infants each year (Moorlag et al. 2019). Based on reports generated in 1931 by Albert Calmette, co-inventor of the BCG vaccine, the average mortality of 8,075 vaccinated infants (1 month to 1 year) who contracted TB was 4.6%, while that of non-vaccinated children of the same age living under comparable conditions was 16% and sometimes surpassed 25% (Calmette 1931). Surprisingly, epidemiological studies conducted

shortly after the BCG vaccine's introduction in the 1920s revealed that it reduced infant mortality independent of its effect on TB (Shann 2010, 2013). According to several observational studies carried out in West Africa, the BCG vaccination reduced overall mortality in children by 50%. The outcome was too noteworthy to be solely explained by immunity to TB (Kristensen et al. 2000; Garly et al. 2003; Roth et al. 2005).

Several studies (Breiman et al. 2004; Aaby et al. 2011, 2015) have found beneficial NSEs of the BCG vaccine on child survival (Roth et al. 2006), with these effects being more noticeable in girls. The original major goal of BCG research, which was carried out in the US and the UK in the 1940s and 1950s, was to prevent TB. When those trials were reanalyzed, it was shown that BCG immunization was associated with a 25% drop in non-TB and non-accident mortality (Shann 2010, 2013). Similar results were discovered in other countries, and more recently, RCTs and a meta-analysis of three RCTs have verified them (Biering-Sørensen et al. 2012; Aaby et al. 2016a; Biering-Sørensen et al. 2017). The BCG-induced reduction in newborn mortality appears to be due to heterologous protection against unrelated infections (Moorlag et al. 2019). In a mouse model, it was discovered that using MTBVAC, a genetically altered version of *Mycobacterium tuberculosis* as a live, attenuated vaccine, produced protection against *Streptococcus pneumoniae* (Tarancón et al. 2020). Randomized controlled trials demonstrated that BCG vaccination is associated with decreased malaria morbidity and mortality in African children (Berendsen et al. 2019; Jensen et al. 2020; Bassat et al. 2020).

The Oral Polio Vaccine (OPV)

At the beginning of the COVID-19 pandemic, it was suggested that live attenuated vaccines, such as the OPV, could boost innate immunity and offer temporary protection against SARS-CoV-2 infection (Chumakov et al. 2020). Albert Sabin created the OPV in the 1950s, composed of three live attenuated polioviruses. Early clinical trials showed that, in addition to preventing poliomyelitis, OPV decreased the number of additional viruses that could be isolated from children who had vaccinations compared to those who received a placebo. Further proof of the non-specific effects of OPV is evident from the Type 1 poliovirus-caused poliomyelitis outbreak in Singapore in 1959, which was effectively contained by monovalent OPV, which only contained Type 2 poliovirus (Hale et al. 1959). In the 1960s and 1970s, extensive clinical research on OPV was conducted for the nonspecific prevention of several diseases.

A study involving more than 60,000 individuals identified OPV as protective against influenza virus infection, reducing morbidity 3.8 times (Voroshilova 1989; Chumakov et al. 1992). In addition to its antiviral effects, OPV also demonstrated antitumor effects by killing tumor cells directly and inducing cellular immunity against them (Voroshilova 1989). Non-pathogenic enteroviruses were isolated from healthy individuals and tested for safety; they later emerged useful as live enterovirus vaccines (LEVs) that significantly lowered the incidence of acute respiratory illnesses and seasonal influenza in over 90,000 people when used for non-specific protection (Voroshilova 1989; Chumakov et al. 1992).

In 1975, an extensive OPV vaccination campaign was launched in Bulgaria to help contain the emergence of an acute poliomyelitis-like disease outbreak unrelated to the Enterovirus 71 (Shindarov et al. 1979). The protective effects of OPV have been proven to be significant, as infant mortality was reduced by around 32% when OPV was given at birth, based on data from a randomized controlled trial (RCT) that took place in Guinea-Bissau, West Africa (Aaby and Benn 2019). One study investigated the impact of annual and biannual nationwide OPV vaccination and found this vaccine led to a 19% decrease in all-cause mortality; subsequent campaigns resulted in another 13% reduction (Andersen et al. 2018).

These findings emphasize the non-specific nature of the OPV-induced protection because they were observed even when there were no poliomyelitis cases (Chumakov et al. 2020). In one RTC, OPV significantly reduced the morbidity of bacterial diarrheal infections among infants in Bangladesh when compared to the Inactivated Polio Vaccine (IPV) (Upfill-Brown et al. 2017). Another study conducted in Finland showed that those children who received OPV had a lower rate of middle ear infections than the IPV-immunized group (Seppälä et al. 2011). An analysis from past studies in Denmark reported that hospital admissions for pediatric respiratory infections were lower when OPV was used (Sørup et al. 2016).

Innate Immune Training as a Mechanistic Framework Explains the Beneficial NSEs of Live Vaccines

Innate immunity and adaptive immunity are the two main categories used for classifying the immune systems of vertebrates. Monocytes, macrophages, neutrophils, dendritic cells (DC), and natural killer (NK) cells are among the cells that compose innate immunity (Netea 2015). These cells are the first system of defense that the host has evolved to recognize pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) through the use of germline-encoded pattern recognition receptors (PRRs), which allow for quick but nonspecific recognition (Netea 2015; Medzhitov 2000). Specialized B and T cells with lifelong immunological memory and selectivity in identifying antigens with high-affinity receptors are part of adaptive immunity (Netea et al. 2016).

For decades, it has been accepted that immunological memory is an exclusive characteristic of the adaptive immune response. This belief has been called into question, nonetheless, by an increasing amount of research showing that innate immune cells and even tissue-resident stem cells can exhibit adaptive properties (Bowdish et al. 2007; Netea et al. 2011; Netea et al. 2016; Naik et al. 2017; Lay et al. 2018). Furthermore, via innate immune processes, some infectious diseases and vaccines can produce widespread protection against other diseases (Netea et al. 2011; Netea et al. 2016). The hypothesis derived from these investigations suggests that the innate immune system also possesses adaptive traits, a feature known as "trained immunity." Understanding the fundamental features of trained innate immunity will aid in our understanding of how the host defends itself and the causes of immune-related diseases (Netea et al. 2020).

Immunological studies of innate immune training are starting to provide potential explanations for the NSEs of vaccines (Aaby et al. 2014; Arts et al. 2018; Chumakov

et al. 2021). Live vaccines induce epigenetic changes that strengthen immunity to unrelated pathogens and instruct the innate immune system. On the other hand, non-live vaccines might encourage "tolerance," which makes kids more vulnerable to infections and associated diseases (Blok et al. 2020). According to a comprehensive compilation of epidemiological data, live vaccines, like the OPV, smallpox, BCG, and measles, have been shown to have positive, non-specific protective effects against diseases other than the disease for which they were created (Aaby et al. 1995, 2006, 2010, 2011, Lund et al. 2015; Biering-Sørensen et al. 2017; Rieckmann et al. 2017; Andersen et al. 2018). Afterward, BCG vaccination trials performed in adults (Kleinnijenhuis et al. 2012, 2014) and children (Jensen et al. 2015; Freyne et al. 2018) showed that this vaccine promotes non-specific activation of innate immune cells.

Both Th1 and Th2 immunity are thought to be implicated in NSEs since an inactivated vaccine like DTP promotes a Th2 profile, whereas a live vaccine like measles induces Th1 immunity (Upfill-Brown et al. 2017). A cohort of healthy individuals who received the BCG vaccine showed higher NK cell and monocyte activation following a controlled human malaria infection (CHMI), associated with a decrease in the number of parasites in their blood. These findings validate the assumption that the BCG vaccine may result in trained immunity with good responses *in vivo* against other human diseases (Walk et al. 2019). Furthermore, it has been demonstrated that BCG vaccination reduces parasitemia in rodent malaria models (Clark et al. 1976; Matsumoto et al. 2000; Parra et al. 2013), and it has been associated with a reduction in malaria mortality in endemic areas (Roth et al. 2005).

Is There Evidence That Pre-existing Vaccines Protected Against Severe SARS-CoV-2 Infection?

The OPV Vaccine

It was hypothesized that repurposing live vaccines could reduce morbidity and mortality from COVID-19 (Chumakov et al. 2020, 2021). Compared to Inactivated Polio Vaccine (IPV)-using countries, OPV-using countries had a reduced incidence of COVID-19. This suggests OPV may help stop SARS-CoV-2 infection at the individual level or slow the virus' spread throughout the population (Habibzadeh et al. 2022). A randomized study was carried out at a Russian medical university. One thousand one hundred fifteen healthy individuals between ages 18 and 65 were randomly assigned to one of two identical cohorts and given either a placebo or a single dosage of bOPV, known as "BiVac Polio." For three months, the study participants' respiratory conditions, especially COVID-19, were observed. After three months of immunization, the endpoint was the frequency of acute respiratory infections and laboratory-confirmed COVID-19 in both groups. Compared to the placebo group, there were much fewer laboratory-confirmed instances of COVID-19 in the immunization group (25 cases vs. 44, $p = 0.036$) (Yagovkina et al. 2022).

After conducting an RCT, it was discovered that during the COVID-19 pandemic, OPV had no overall beneficial effect on severe morbidity in persons older than 50 years (Fisker et al. 2022). Previous epidemiological research has documented the

advantageous heterologous pan-viral effect of OPV (Chumakov et al. 1992; Lund et al. 2015), and observational studies also pointed to a potential influence on COVID-19. Compared to age-matched women, mothers of children who received the OPV vaccination also showed a lower vulnerability to SARS-CoV-2 infection. In this instance, the mother's exposure to the OPV virus from the infants' feces would probably have protected them (Habibzadeh et al. 2021). When children get this vaccine, they can shed the weakened virus in their stools for several weeks. This means that if a person comes into contact with the stool of a vaccinated child (for example, mothers taking care of young infants), such an individual could be exposed to the live, weakened poliovirus. Mothers have a higher chance of contracting the virus because they handle their children's hygiene (Habibzadeh et al. 2021).

However, a study found that the COVID-19 disease impact, expressed as the overall number of COVID-19-confirmed cases and death rates per million people, was higher in nations with higher rates of polio vaccination coverage (Afify et al. 2021). These authors concluded that the goal of polio vaccination campaigns should only be restricted to eliminating the illness from endemic nations, yet there is no conclusive proof that live-attenuated vaccinations protect against severe COVID-19 (Afify et al. 2021). Although the reviewed literature reported some protection levels against SARS-CoV-2 infection, such protection was not enough to reduce disease severity and mortality.

The BCG Vaccine

It was suggested that the epidemiology of COVID-19 may be impacted by a nation's BCG immunization regulations (Miller et al. 2020). This hypothesis was mostly supported by the observation that nations without a mandatory BCG vaccination policy, like Italy, were severely impacted by the SARS-CoV-2 outbreak even though they took extensive social isolation measures, whereas countries that have the immunization policy, like Japan, were only marginally affected (Miller et al. 2020). However, that conclusion was not supported by a study performed on patients diagnosed with COVID-19 pneumonia between March and June 2020 in a state hospital in Turkey, a country that implemented a BCG vaccination program in 1953 (Aksu et al. 2020). The patients' diagnosis was supported by pulmonary infiltrates on computed chest tomography and positive results for the coronavirus-2 polymerase chain reaction in a nasopharyngeal sample. Also, 123 persons between the ages of 19 and 87 were diagnosed with COVID-19 pneumonia. Thirty-four participants (27.6%) and 89 subjects (72.4%), respectively, had moderate and severe pneumonia within the study population. Ninety-one patients (74.0%) had received the BCG vaccination. Results showed that the severity of COVID-19 pneumonia was more strongly associated with being older and having a lower income than whether or not someone received the BCG vaccine (Aksu et al. 2020). After controlling for confounding variables, several investigations discovered that there was no longer any correlation between the BCG vaccination policy and the COVID-19 spread rate or death rate (Hensel et al. 2020; Wassenaar et al. 2020; Chimoyi et al. 2020).

In 2020, other researchers evaluated the hypothesis that the BCG vaccine was associated with better clinical outcomes in COVID-19 patients. They analyzed 55

countries that meet the criteria for population size and the number of COVID-19 deaths per million people (DPM). Countries that have been administering the BCG vaccine for a long time tended to have fewer DPMs from COVID-19. The results were corroborated by permutation tests. It was suggested that BCG vaccination coverage, particularly among the recently vaccinated population, may help lessen the propagation of the virus and reduce the severity of infection (Klinger et al. 2020). Furthermore, researchers investigated different age cohorts in various countries to reveal how BCG vaccination coverage was associated with COVID-19 deaths. They found that the strongest correlation between vaccination and lower death rates was in the younger population, specifically those aged 0–24 years; interestingly, the association was relatively small and insignificant for the older age group (>65 years) for all investigated timeframes (Klinger et al. 2020). The significance of the young group age in the disease's dissemination was confirmed by epidemiological research conducted on COVID-19-positive individuals in Shenzhen, China (Bi et al. 2020). Children between the ages of 1 and 16 were specifically recognized to be essential to the transmission chain (Kelvin and Halperin 2020). Overall, findings from that research (Klinger et al. 2020) suggest that the reduction in COVID-19 deaths was not mediated by an enhancement in innate immunity but rather by interfering with the SARS-CoV-2 transmission chain.

In a randomized, placebo-controlled research, healthcare professionals were allocated to receive either the BCG-Denmark vaccine or saline placebo and were monitored for an entire year. The major outcomes, symptomatic and severe COVID-19, were evaluated at 6 months. Healthcare professionals who received the BCG-Denmark vaccine did not have a decreased risk of COVID-19 compared to those who did not (Pittet et al. 2023).

The effects of BCG can also vary among pathogens (Kaufmann et al. 2022). For instance, a newborn's protection against TB, respiratory tract infections, neonatal sepsis, and all-cause mortality is linked to the BCG vaccination (Aaby et al. 2011; Rieckmann et al. 2017). Furthermore, OPV has an additional favorable effect on mortality, as both the BCG and polio vaccinations at birth result in 32% reduced mortality than the BCG immunization alone (Lund et al. 2015). Preclinical evidence in murine infection models corroborates these findings. It has been demonstrated that BCG vaccination confers protection against parasites (e.g., *Schistosoma mansoni*) (Tribouley et al. 1978), fungal infections (e.g., *Candida albicans*) (Van't Wout et al. 1992), DNA and RNA viruses (e.g., herpes and influenza virus) (Spencer et al. 1977; Hippmann et al. 1992; Mukherjee et al. 2017). Furthermore, BCG vaccination has been linked to immunity against human experimental models of malaria (Walk et al. 2019) and yellow fever (Arts et al. 2018), as well as all-cause respiratory infections in older adults (Giamarellos-Bourboulis et al. 2020). These findings have been reported in healthy adults. Therefore, the cross-protective benefits of BCG vaccination are supported by epidemiological research and pre-clinical and clinical data (Namakula et al. 2020).

However, most of the larger investigations conducted in South Africa and Europe did not demonstrate a protective effect of BCG vaccination against the susceptibility to COVID-19, regardless of whether the research was performed in older people who are more susceptible to severe infections (Moorlag et al. 2022) or

in healthcare workers (Ten Doesschate et al. 2022; Upton et al. 2022; Czajka et al. 2022;Pittet et al. 2023).

Discussion

A painful lesson that needs to be addressed from the COVID-19 pandemic is that approximately 3,000,000 people died during the first year because there was no specific vaccine against SARS-CoV-2. At the onset of the COVID-19 pandemic, there was heightened interest in evaluating the effects of existing vaccines and trained innate immunity induction on SARS-CoV-2 infection due to the potential of certain vaccines, particularly those containing live attenuated microorganisms, to provide substantial heterologous defense against this pathogen (O'Neill and Netea 2020). Although several studies with small susceptible populations demonstrated protective benefits, most investigations in healthy individuals did not show significant protection against severe COVID-19. Several of these vaccines were suggested to elicit a decrease in illness severity, albeit this was not convincingly proved (Netea et al. 2023). The heterologous protection against other pathogens induced by live attenuated vaccines has been demonstrated for decades. However, it was not enough to protect against severe SARS-CoV-2 infection. Why?

An adage created by the Chinese military general Sun Tzu (Tzu 2008) says: "Attack is the secret of defense; defense is the planning of an attack." This work proposes that SARS-CoV-2 (in addition to evasion mechanisms) employs offensive strategies instead of just waiting to be attacked.

Recent investigations demonstrated that BCG-induced trained innate immunity occurs in the bone marrow (BM) of both people and animals against tuberculosis (TB) (Kaufmann et al. 2018; Cirovic et al. 2020). According to contemporary findings in a mouse model of TB, the BCG vaccine-induced protective trained innate immunity by reprogramming BM-resident hematopoietic stem cells (HSCs) toward myeloid cell production in an interferon-gamma (IFN-γ)-dependent pathway (Kaufmann et al. 2018). Interestingly, it was also shown that trained innate immunity can be inhibited by *Mycobacterium tuberculosis* (Khan et al. 2020). Specifically, within 10 days of the infection, *M. tuberculosis* spreads from the lungs to the BM. Once there, this bacterium alters the Type I IFN/iron axis to preferentially cause a type of cell death called necroptosis, specifically in the myeloid cells. Simply put, the bacterium sabotages the immune system's response by killing these important cells (Khan et al. 2020).

In the case of COVID-19, there are reports of the presence of SARS-CoV-2 in the blood, a condition known as viremia (Puelles et al. 2020; Di Cristanziano et al. 2021) and viral RNA found in the BM of COVID-19 patients (Deinhardt-Emmer et al. 2021; Jurek et al. 2022) and mice (Kaufmann et al. 2022). Therefore, it was hypothesized that BCG-mediated trained immunity against SARS-CoV-2 is compromised by viral dispersion to the BM due to pulmonary vascular injury (Kaufmann et al. 2022). While BCG immunization greatly decreased morbidity and mortality from influenza A virus (IAV) in mice and hamsters, it did not protect against severe SARS-CoV-2 infection. Unlike IAV, SARS-CoV-2 infection causes distinct damage to the pulmonary vasculature, which makes the virus easier to spread

to other organs, such as the BM, which is a key location for BCG-mediated trained innate immunity (Kaufmann et al. 2022). It could be thought that SARS-CoV-2 induces such damage to the lung tissue to get access to systemic blood circulation and favor its dissemination to other organs, such as the BM.

Type I interferons (IFNs) are essential elements of the early antiviral response that limit the replication and spread of viruses utilizing autocrine and paracrine Type I IFN receptor (IFNAR) signaling. However, in patients with severe COVID-19, tiny amounts of IFNs have been found in the lungs or peripheral blood (Blanco-Melo et al. 2020; Hadjadj et al. 2020). In particular, the SARS-CoV-2 infection results in an insufficient and delayed IFN-I response, which is thought to be the primary factor causing the severity of the disease (Bastard et al. 2020; Lei et al. 2020; Hadjadj et al. 2020; Blanco-Melo et al. 2020; Trouillet-Assant et al. 2020; Galani et al. 2021). Several viral proteins are responsible for this impairment (for review, see ref. Rubio-Casillas et al. 2022).

Because it is more advantageous to strike first rather than wait to be attacked, SARS-CoV-2 strategically anticipates and affects leukocyte production in the BM. Several studies have reported marked lymphopenia in severe cases of COVID-19 (Gutiérrez-Bautista et al. 2020; Han et al. 2020; Shi et al. 2020; Wang et al. 2020; Bo et al. 2020; Cantenys-Molina et al. 2021; Gil-Etayo et al. 2021). A reduction in T cells was present in about 85% of hospitalized patients (Gutiérrez-Bautista et al. 2020; Lafarge et al. 2021), while in mild COVID-19 cases, the lymphocyte count stayed within the normal range (Liu et al. 2020). One of the potential causes of lymphopenia is BM suppression (Gutiérrez-Bautista et al. 2020). It has been suggested that SARS-CoV-2-induced chemokines suppress the survival of HSCs in the BM (Chi et al. 2020). In one study, BM mononuclear cells from COVID-19 patients were evaluated using single-cell RNA sequencing, while peripheral blood mononuclear cells served as the control. The findings demonstrated that HSCs from these patients were mostly in the G1 phase and were at risk for apoptosis. In the most severe cases, there was a marked decrease in lymphoid progenitors and a notable concentration of immature myeloid progenitors (Wang et al. 2021). SARS-CoV-2 infection also causes modifications such as a lower-than-normal number of blood cells (pancytopenia), phagocytosis of precursor cells (hemophagocytosis), and BM necrosis (Debliquis et al. 2020; Zeylabi et al. 2023; Marques-Maggio et al. 2023).

The thymus is also affected by SARS-CoV-2 infection (Ciofani and Zúñiga-Pflücker 2007; Rosichini et al. 2023). It is well known that lymphocytes, like all other hematopoietic cells, are produced from HSCs found in the BM, but contrary to most blood cells, the majority of T cell development and training takes place in the thymus (Ciofani and Zúñiga-Pflücker 2007). A study was carried out on adult patients hospitalized in Italy from early March to October 2020 (Rosichini et al. 2023). The expression of ACE2 receptors, which allows SARS-CoV-2 to infiltrate host cells, was present in the thymic epithelium. It was found that patients with COVID-19 showed a significant decrease in thymic T-cell survival, and the decline in thymic function was associated with the severity of the disease (Rosichini et al. 2023).

Interestingly, in the animal model, the Delta variant of SARS-CoV-2 induced the most severe thymic atrophy, marked by significantly reduced T cell numbers (due to apoptosis), whereas Omicron only produced marginal thymic atrophy. This study

suggested that thymic dysregulation and thymic atrophy could be responsible for the lymphopenia linked to severe SARS-CoV-2 infection (Rizvi et al. 2024).

Through natural selection, SARS-CoV-2 has developed sophisticated attack and evasion techniques, enabling it to escape immune surveillance and destruction, demonstrating that the best defense is a potent offense. The virus sabotages two important immune organs: the BM and the thymus. It is proposed that this accurate attack (inhibiting innate and adaptive immune training) is why vaccines such as OPV and BCG could not reduce the severity of infection and mortality from COVID-19.

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